211487

QUALITY ASSURANCE PROJECT PLAN

FOR

Vasquez Blvd-I70 Bioavailability of Arsenic in Site Soils Using Juvenile Swine as an Animal Model

September 1999

Prepared with technical assistance by:
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Denver, CO

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Program Approval date
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A3 Distribution List

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A4. PROJECT TASK ORGANIZATION

A4.1 Project Task

EPA Region 8 is seeking to characterize the bioavailability of arsenic in fine particulate surface soils at the Vasquez Boulevard and I-70 (VB-I70) Study Area using juvenile swine as an animal model. This document serves as the Biological Media Sampling and Analysis Plan and Quality Assurance Project Plan (QAPP) for the project and presents the organization, objectives, functional activities and specific quality assurance and quality control activities associated with the bioavailability investigation. This QAPP includes study background information, project objectives and scope, analytical design and rationale, and data quality objectives (DQOs). It describes the specific protocols that will be followed for obtaining study materials, implementing the study, processing and storing of biological samples, preparing chain of custody forms, and conducting laboratory analyses. Surface soil (test substance) sampling, handling and partial analysis can be found in the *Pilot Soil Characterization Study* (EPA, 1999).

A4.2 Project Organization

The following lists key personnel who will serve as contacts and provide technical expertise during implementation of this Project Plan along with their designated roles and responsibilities.

Bonita Lavelle, EPA Remedial Project Manager, will be responsible for overall project management and coordination among EPA and its contractors and other interested parties.

Christopher P. Weis, Ph.D., DABT. EPA Regional Toxicologist, will serve as the study design advisor and science manager for this project.

Stan Casteel, DVM, PhD, Principal Investigator, will be responsible for implementing and documenting all activities associated with dosing animals and collecting samples.

William Brattin, Ph.D., ISSI, Inc., will be responsible for technical management of ISSI's activities which include: preparing planning documents, providing technical oversight, and compiling and summarizing data generated during the investigation.

Tracy Hammon, M.S., ISSI, Inc., will be responsible for preparation of study investigation materials including; chain of custody forms, time details and dosing spreadsheets. In addition, Ms. Hammon will perform the data reduction for results from this study and calculate a bioavailability value for arsenic in juvenile swine.

Mary Goldade, M.S., ISSI, Inc., will serve as the QA officer for ISSI's role in this project.

John Drexler, Ph.D., University of Colorado, will be responsible for preparing samples for analysis and for performing analytical measurements of surface soil samples for metals, phase speciation and in vitro bioaccessibility.

A5 PROBLEM DEFINITION and BACKGROUND

A5.1 Background

The VB-I70 study area is located north of downtown Denver in the state of Colorado. Due to the discovery of sporadic elevations in surficial arsenic levels, recent investigations have been initiated to determine the nature, extent, and public health implications of these findings in the residential areas of the site.

The Colorado Department of Public Health and Environment (CDPHE) collected approximately twenty-five soil samples from residential yards in the Vasquez Blvd-I70 study area during the summer of 1997. Samples were collected from yards north of Interstate 70 in the Swansea and Elyria neighborhoods. The samples indicated levels of arsenic from 12 to 1,300 mg/kg, and lead from 61 to 660 mg/kg. This discovery prompted further investigation to determine the extent of arsenic and lead present in this area.

During the spring of 1998, the USEPA Superfund Technical Assessment and Response Team (START) under contract 68-W5-0031 conducted further sampling and analysis in the area. Samples were again collected from residences in the Elyria and Swansea neighborhoods bounded by Colorado Boulevard on the east, the South Platte River on the west, 38th Avenue on the south, and 56th Avenue on the north. An additional 1200 residences were sampled, identifying 207 properties with arsenic greater than 70 mg/kg and 77 properties with lead greater than 500 mg/kg (UOS, 1998). Sampling efforts to date are continuing southward until the areal extent of the contamination is clearly defined. Residential arsenic concentrations in these follow-up investigations ranged higher than 10,000 mg/kg in selected yards.

A number of soils from this study area have undergone further characterization for metal speciation using electron microprobe analysis and *in vitro* bioaccessability (solubility testing). Arsenic found in soils at the VB/I-70 site was determined to be primarily in the form of arsenic trioxide (>95%). While *in vitro* solubility tests are presently experimental and therefore unsuitable for site specific adjustments in bioavailability, the tests indicated that solubility of the arsenic forms found in the VB/I-70 soils might be lower than expected. Based on the results of this further characterization, EPA will evaluate the *in vivo* bioavailability of arsenic in study area soils using juvenile swine as an animal model. This information will be used to help evaluate the potential risk to residents from exposure to arsenic in site soils.

A5.2 Problem Definition – Conceptual Model

An as yet unidentified source(s) has led to elevated residential soil concentrations of lead and arsenic, resulting in Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or Superfund) actions by the Environmental Protection Agency (EPA) to assess and abate these hazards to human health and the environment. Accurate assessment of the human health risks resulting from oral exposure to metals requires knowledge of the amount of metal absorbed from the gastrointestinal tract into the body. This information is especially important for environmental media such as soil or metal extraction industry wastes, because metals in these media may exist, at least in part, in a variety of poorly water soluble minerals, and may also exist inside particles of inert matrix such as rock or slag. These chemical and physical properties may tend to influence (usually decrease) the absorption (bioavailability) of the metals when ingested. Therefore, reliable site-specific data on metal bioavailability in environmental media of concern may be expected to increase the accuracy and decrease the uncertainty in human health risk estimates. Preliminary *in vitro* bioaccessability testing on arsenic in site soils show low bioaccessability (solubility) values ranging from 3 - 26% as compared to EPA Region 8's default value of 80% for inorganic arsenic contamination in soils. In order to obtain more reliable information on the actual *in vivo* absorption of arsenic in these soils, EPA will run an *in vivo* study for arsenic bioavailability in juvenile swine as a plausible surrogate for arsenic absorption by humans.

This project plan will describe the efforts planned by EPA to evaluate the bioavailability of arsenic in soils from the study area using juvenile swine as an animal model. The overall approach will follow the methods developed by the EPA Region 8 and employed in the Phase II Bioavailability Studies (EPA, 1995).

A6 PROJECT TASK DESCRIPTION

A6.1 Study Goals

The study goal is to collect data that will allow a plausible estimate of *in vivo* relative arsenic absorption from site-specific soils when compared by statistical and biological means to arsenic absorption from a freely soluble arsenic form (sodium arsenate). This estimate will be used for risk assessment and possibly risk-based decision-making for the human health risk assessment at the VB/I-70 site.

In order to accomplish this goal the following general and specific quality objectives have been defined.

A6.2 Study Objectives

General Objective #1:

to determine quantitatively whether VB/I-70 soil arsenic is absorbed to a lesser or greater extent than freely soluble arsenic in water; and

General Objective #2:

to estimate a site specific absorption fraction for soil arsenic which is protective and plausibly applicable for human health risk assessment at the VB/I-70 site.

A7 QUALITY OBJECTIVES and CRITERIA for MEASUREMENT DATA

The Data Quality Objectives (DQO) process is an iterative process which is designed to focus on the decisions that must be made and to help ensure that the site activities acquire data that are logical, scientifically defensible, and cost effective. The DQO process is intended to:

- Ensure that task objectives are clearly defined;
- Determine anticipated uses of the data;
- Determine what environmental data are necessary to meet these objectives; and
- Ensure that the data collected are of adequate quantity and quality for the intended use.

A7.1 Study Objective DQOs

Two types of objectives are identified in this QAPP: general objectives and data quality objectives (DQOs}. General objectives are statements of practical goals that, if realized, will substantially contribute to achieving the purpose of the study. Development of DQOs is a process that is intended to ensure that task objectives are clearly defined and that data collected are appropriate and of sufficient quality to satisfy the objectives. DQOs for each of the study objectives are provided below.

<u>General Objective #1:</u>

to determine quantitatively whether VB/I-70 soil arsenic is absorbed to a lesser or greater extent than freely soluble arsenic in water:

General Objective #2:

to estimate a site specific absorption fraction for soil arsenic which is protective and plausibly applicable for human health risk assessment at the VB/I-70 site.

<u>Specific Data Quality Objective Process</u>

The three stages of the DQO process are identified below and a discussion of how they have been applied in the study described herein. The three stages are undertaken in an interactive and iterative manner, whereby all the DQO elements are continually reviewed and re-evaluated until there is reasonable assurance that suitable data for decision making will be attained.

- <u>Stage I - Identify Decision Types</u>: Stage I defines the types of decisions that will be made by identifying data uses, evaluating available data, developing a conceptual model, and specifying objectives for the project. The conceptual model facilitates identification of decisions that may be made, the end use of the data collected, and the potential deficiencies in the existing information.

- <u>Stage II - Identify Data Uses/Needs</u>: Stage II stipulates criteria for determining data adequacy. This stage involves specifying the quantity and quality of data necessary to meet the Stage I objectives. EPA's Data Useability for Risk Assessment Guidance (DURA) outlines general and specific recommendations for data adequacy. This includes identification of data uses and data types, and identification of data quality and quantity needs.

- <u>Stage III - Design Data Collection Program</u>: Stage III specifies the methods by which data of acceptable quality and quantity will be obtained for use in decision making. These methods are provided in the attached SOPs.

Through utilization of the DQO process, as defined in EPA guidance (EPA540-R-93-071 and -078, Sep 1993), this QAPP will use several terms that are specifically defined to avoid confusion that might result from any misunderstanding of their use. For each of the tasks identified within this QAPP, a "Task Objective" is specifically defined. The Task Objective is a concise statement of the problem to be addressed by activities under this task. For each Task Objective, a decision (or series of decisions) is identified which addresses the problem contained in the Task Objective.

For each decision, the data necessary to make the decision are identified and described. For all analytical data, quality assurance objectives are specified that describe the minimum quality of data necessary to support the specified decision or test the hypotheses. These quality assurance objectives are specified as objectives for precision, accuracy, representativeness, comparability, and completeness. In addition, data review and validation procedures are specified in the QAPP that evaluate how well the analytical data meet these quality assurance objectives and whether or not the data are of sufficient quality for the intended usage.

The following sections apply the DQO process to the North Denver, Colorado Response, Stage I and Stage II, where Stage I and Stage II identify decision types and data uses/needs for the SAP. Stage III is discussed later and provides the specific task objectives, decisions, and rationale for resolving the decisions necessary to complete this Study.

DQO Stage I - Identifying Decision Types

Stage I of the DQO process identifies a primary question and secondary questions that need to be resolved at the completion of the sampling and analyses program.

- PRIMARY QUESTION 1: is VB/I-70 soil arsenic absorbed to a lesser or greater extent than freely soluble arsenic (sodium arsenate, NaAs)?

- PRIMARY QUESTION 2: is the data of sufficient quantity and quality to estimate a plausible value for relative arsenic bioavailability?

DQO Stage II - Identifying Data Uses/Needs

Stage II of the DQO process identifies data uses and needs. The primary uses of data are:

- Compare data from site test materials to data from a control material to develop a quantitative relative estimate of the bioavailability of soil arsenic when compared (using standard statistical analyses) to freely soluble arsenic.

- Using relative absorption data derived from the *in vivo* study, estimate a site specific relative bioavailability (RBA) for soil arsenic which is protective of human health and plausibly applicable for human health risk assessment at the VB/I-70 site.

In order to accomplish these uses, sample collection will be designed to ensure: 1) sufficient soil samples are tested during the course of the investigation; 2) that these soil samples are fully characterized to estimate their representativeness of arsenic at the site; 3) that sufficient biological samples are collected to support standard statistical comparison between dose groups and test substances; 4) that collection of biological samples is random within the study design; and 5) that sample handling and labeling ensures that analysis will be blind and otherwise according to Good Laboratory Practices of EPA..

Stage II of the DQO process also determines what type and quality of data are needed to answer the questions developed in Stage I. Within this QAPP, quantitative and qualitative limits are defined for precision, accuracy, representativeness, comparability and analytical completeness. Reporting limits for chemical analytes are set by the analytical laboratory based on matrix, historical data, and comparison to EPA limits for CLP and other methods. Quantitative limits are also defined for instrument and method detection limits, and for method reporting limits or method quantitation limits. The QA procedures outlined in this section are intended to ensure data quality and to administer corrective actions with the goal of producing data that satisfy the following requirements. General guidelines, policies, and procedures to achieve these objectives are presented below. Where additional, detailed, procedures are required to attain QA objectives and to describe specific methods, these are provided in the attached SOPs. The following PARCC requirements apply to more standard chemical analytical analyses:

Precision: Precision is defined as the agreement between a set of replicate measurements without assumption or knowledge of the true value. It is a measure of agreement among individual measurements of the same property under prescribed similar conditions. Agreement is expressed as either the relative percent difference (RPD) for duplicate measurements or the range and standard deviation for larger numbers of replicates. The RPD will be reported on required 5% laboratory duplicates, and a defined MDL will be reported as per EPA guidance in CFR, part 136, app. B (7 method-replicates on 3 non-consecutive days of a low-level [near MQL] standard, with MDL = 3 x SD).

Study personnel will prepare blind duplicate samples. A minimum of one blind duplicate will be prepared for 5-10% of the samples collected. These blind duplicate samples will be specified in the study design.

Accuracy: Accuracy is a measure of the closeness of individual measurements to the "true" value. Accuracy usually is expressed as a percentage of that value. For a variety of analytical procedures, standard reference materials traceable to or available from National Institute of Standards and Technology (NIST, formerly National Bureau of Standards) or other sources can be used to determine accuracy of measurements. Accuracy will be measured as the percent recovery (%R) of an analyte in a reference standard or spiked samples (>3) that span the limit of linearity for the method.

Ideally, precision and accuracy estimates should represent the entire measurement process, including sampling, analysis, calibration, and other components. From a practical perspective, these estimates usually represent only a portion of the measurement process that occurs in the analytical lab.

Representativeness: Representativeness is the degree to which data accurately and precisely represent characteristics of a population, parameter variations at a sampling point, or an environmental condition. For this QAPP, samples representative of soils in the study area are to be selected from those previously tested for speciation and bioaccessibility.

Comparability: Data are comparable if study considerations, collection techniques, and measurement procedures, methods, and reporting are equivalent for the samples within a sample set. A qualitative assessment of data comparability will be made of applicable data sets. These criteria allow comparison of data from different sources. Comparable data will be obtained by specifying standard units for physical measurements and standard procedures for sample collection, processing, and analysis.

Completeness: Data are considered complete when a prescribed percentage of the total intended measurements and samples are obtained. Analytical completeness is defined as the percentage of valid analytical results requested, and >90% of analyzed samples should have results reported. For this sampling program, a minimum of 80 percent of the planned collection of individual samples must be obtained to achieve a satisfactory level of data completeness.

Method Detection Limits (applicable to chemical analyses only): Method detection limits (MDLs) are minimum values that can be reliably measured to identify the analyte as being present in the matrix, vs method quantitation limits are the minimum values that can be quantitated with reasonable scientific confidence. The method will also have a maximum linear value in most situations, and analyses should occur within this limit of linearity range.

B. MEASUREMENT AND DATA ACQUISITION

B1 SAMPLING PROCESS DESIGN

This section provides an overview of the methods to be used in determining bioavailability of arsenic in site soils. Detailed protocols are provided in the attached SOPs.

The USEPA has been engaged in a multi-year investigation of the bioavailability of metals in soil and mine waste. This study has focused mainly on lead (Weis and LaVelle, 1991; Weis et al, 1994;, Casteel et al., 1997) but a number of studies were performed to investigate the relative bioavailability (RBA) of arsenic in a variety of test materials. This study will follow the sampling methods developed by EPA in previous studies.

Three representative site soil samples will be selected for inclusion in this study. These samples will be fully characterized to determine their physico-chemical characteristics including arsenic concentrations and to assess their representativeness for the site as a whole. The samples will then be administered to juvenile swine using a daily dosing protocol. Urine samples will be collected and analyzed for arsenic in order to determine the relative amount of arsenic absorbed from the animal groups dosed with soil vs animal groups dosed with freely soluble arsenic in the form of sodium arsenate (NaAs).

This study will be performed using young swine as the test species because the gastrointestinal system of swine is more nearly similar to humans than most other animal models. The animals will be housed individually in metabolic cages (cages designed to collect and separate urine and feces). Groups of randomly selected animals (N= 4) will be given oral doses of test material or sodium arsenate (NaAs) for a total of 12 days, with the dose for each day being administered in two equal portions given at 9:00 AM (after an overnight fast) and 3:00 PM (two hours before feeding). Doses will be based on measured group mean body weights, and will be adjusted every three days to account for animal growth.

The test materials have been intentionally left unidentified in this project plan so that the plan may be used for multiple studies of test materials from this site. A memo documenting specific test materials will be prepared prior to the commencement of each study. All test materials which are used in the swine bioavailability study will undergo characterization and *in vitro* solubility testing. Characterization will include CLP metals analysis according to EPA method SW-846, evaluation of soil pH according to EPA method 9045C, measurement of total organic carbon according to EPA method 9060 and metals speciation according to SOP ISSI VBI70-09 (note: perlite will not be quantified). *In vitro* testing will be performed according to the SOP in Appendix A.6 in the Pilot-Soil Characterization Plan for this site (attached as SOP #16) (EPA, 1999).

For animals exposed by the oral route, dose material will be placed in the center of a small portion (about 5 grams) of moistened feed (referred to as a "doughball"), and administered to the animals by hand. All missed doses will be recorded and the time-weighted average dose calculation for each animal will be adjusted downward accordingly.

The following table shows the study design for evaluating the bioavailability of arsenic in site soils.

Group	Number of Animals	Material Administered	Dose Route	Dose (ug As/kg-day)
1	3	Control	Oral	0
2	4	NaAs	Oral	50
3	4	NaAs	Oral	125
4	4	Test Material #1	Oral	50
5	4	Test Material #1	Oral	125
6	4	Test Material #2	Oral	50
7	4	Test Material #2	Oral	125
8	4	Test Material #3	Oral	50
9	4	Test Material #3	Oral	125

Samples of urine and feces (48 hour composites) will be collected from each animal on days 6-7, 8-9, 10-11 during the study. Each collection of urine will be conducted by placing a stainless steel pan beneath each cage, which drains into a plastic storage bottle. Each collection pan will be fitted with a nylon screen to minimize contamination with feces, spilled food, or other debris. Plastic diverters will be used to minimize urine dilution with drinking water spilled by the animals from the watering nozzle into the collection pan.

Aliquots of the urine and feces samples will be analyzed for total arsenic content. Measurement of urinary arsenic concentrations provides a measure of the amount of arsenic which was absorbed by the animal, whereas measurement of arsenic in feces provides a measure of the amount of arsenic which was not absorbed by the animal.

The amount of arsenic absorbed will be evaluated by measuring the amount of arsenic which was excreted in urine. The amount excreted in the urine can be expressed as the URINARY EXCRETION FRACTION (UEF). This is estimated by plotting mass recovered in urine per 48 hours divided by the amount given per 48 hours. The ratio of the urinary excretion fraction for some test material (e.g., arsenic in site soil) compared to the urinary excretion fraction for some readily absorbable form of arsenic (e.g., sodium arsenate) is a measure of the RELATIVE BIOAVAILABILITY (RBA):

$$RBA = UEF(test) / UEF(NaAs)$$

An RBA value of 1.0 means that arsenic in the test soil is just as well absorbed as sodium arsenate. An RBA value of 0.5 means that arsenic in the test soil is absorbed 50% as well as sodium arsenate.

The site-specific RBA is used to adjust the toxicity factors for arsenic as follows:

RfD (adjusted) = RfD (default) / RBA
Oral slope factor (adjusted) = Oral slope factor (default) * RBA

B2 SAMPLING METHODS REQUIREMENTS

The proposed sampling consists of the collection of approximately 105 samples of urine from exposed or control animals.

QA/QC samples will consist of blind spikes, media blanks and duplicate samples at a 5-10% rate, and measures of arsenic in other media to which the swine are exposed (e.g., water, feed). Every reasonable effort will be made to adhere strictly to specified TSOPs and Good Laboratory Practices as outlined by EPA in 40 CFR 792. Where deviation from TSOPs and/or GLP guidelines is unavoidable, documentation of the deviation and its potential impact on the outcome of the data collection effort will be documented. Detailed logbook notes will record information pertinent to each sample collection. These notes will be indexed and made available for review following sample collection.

B3 Sampling, Handling and Custody Requirement

Documentation of sample collection, handling, and shipment will include completion of chain-of-custody forms, use of time details and prepared forms, and entry of data and/or observations into a logbook. A chain-of-custody form shall accompany every shipment of samples to the analytical laboratory. The purpose of the chain-of-custody form is to establish the documentation necessary to trace possession from the time of collection to final disposal.

The chain-of-custody form will have the following information:

- Project number
- Sampler's signature
- Date of sample collection
- Collection Media (e.g., Urine)
- Sample identification number
- Analytical parameters

The shipping forms or transmittal memo will describe:

- Number of containers
- Sample preservative (dry ice for transit)

- Date and time of sample shipments

The labs will enter the following information upon receipt:

- Name of person receiving the sample
- Date of sample receipt
- Sample condition

All corrections to the chain-of-custody record will be **initialed and dated by the person making the corrections**. Each chain-of-custody form will include signatures of the appropriate individuals indicated on the form. The originals will accompany the samples to the laboratory, and copies documenting each custody change will be recorded and kept on file.

Chain-of-custody will be maintained until final disposition of the samples by the laboratory and acceptance of analytical results by EPA. One copy of the chain-of-custody will be kept by field personnel.

All required paper work, including sample container labels, chain-of-custody forms, custody seals and shipping forms will be fully completed in ink prior to overnight shipping of the samples to the laboratory.

Upon receipt, coolers containing the biological samples will be received by the laboratory sample custodian. The coolers will be opened and the contents inspected. Chain-of custody forms will be reviewed for completeness, and samples will be logged and assigned a unique laboratory sample number. Any discrepancies or abnormalities in samples will be noted.

The EPA Project Manager will maintain original log books and receive all data packages and reports.

B4 ANALYTICAL METHODS REQUIREMENTS

See the attached laboratory SOP for analytical methods and requirements.

B5 Quality Control Requirements

The project team organization ensures attainment of QA objectives by:

- Assigning responsibility for performing work according to specifications
- Providing oversight of quality-related activities for verification of conformance with specifications
- Defining the relationships between management and personnel performing quality-related work Corrective Action

The Project Manager and Regional Toxicologist will prepare a summary of quality-related activities and problems. This summary will be forwarded to EPA for inclusion in the project file. If deficiencies in the program are identified, the Regional Toxicologist, in consultation with the Project Manager will identify recommendations for corrective action.

Communications. Lines of communication between project personnel and project management staff will be appropriate to enable timely response to events that have the potential to affect data quality. Project personnel will be provided with a project contact list that includes telephone numbers for both routine communications and emergency notifications.

Communications will also entail ensuring that information on sample collection, transportation, analysis, and storage; data acquisition, analysis, and reporting; personnel assignments and activities; and other information pertinent to the project are distributed to potentially affected personnel in a timely manner. Changes in procedures, equipment, personnel, or other program elements as a result of an accident or emergency that have the potential to affect data quality or achievement of overall program objectives will be communicated to the Project Manager in writing in a timely manner.

Copies of all written communications and written summaries of all substantive telephone conversations will be placed in a permanent project file maintained by the EPA Project Manager.

Laboratory Responsibilities. The laboratory and its staff will have the responsibility for processing all samples submitted according to the specific protocols for sample custody, holding times, analysis, reporting, and associated laboratory QA/QC. Laboratory spikes, duplicates, etc. will be performed.

B7 INSTRUMENT CALIBRATION and FREQUENCY

SOPs will identify requirements needed to be met by the field staff and laboratories to meet adequate instrument calibration frequency, and QA/QC for raw data and reports.

C. ASSESSMENT OVERSIGHT

C1 ASSESSMENTS and RESPONSE ACTIONS

The Principal Investigator will be on-site to oversee, implement and inspect study activities associated with the in life stages of the project. Enough sample will be taken and archived to allow for problems (such as loss or spoilage) from transportation or analytical labs.

D. DATA VALIDATION and USABILITY

D1 DATA REVIEW, VALIDATION and VERIFICATION REQUIREMENTS

Data validation will consist of a) establishing an absolute range, acceptance limits (screening criteria), and appropriate statistics for each data parameter, b) describing methods for determining the disposition of suspect data, and c) documenting final disposition of invalid or qualified data, including outliers.

Test Statistic: Qualitative professional judgement will be used to interpret the results of the chemical and biological data collected which is intended to be screening-level preliminary data.

Out-of-range chemical data will be excluded from the validated data set unless the appropriate data value can be positively established and documented. Other suspect data or samples will be examined in detail, including any irregularities in its collection and handling. In the absence of any clear indication that they are invalid (such as equipment failure or operator error), data outliers will remain in the validated data set but will be flagged as outliers per specified criteria (e.g., >3 x SD from the mean). Data points determined to be invalid will be permanently flagged in a clear and consistent manner in the original raw data set and removed from subsequent data summaries and files.

QA for data validation will ensure that the screening criteria are comprehensive, unambiguous, reasonable, and internally consistent; and that data validation activities are properly documented. Data discrepancy reports should be prepared describing any data problems observed and any data correction activities undertaken.

All data records should be cataloged and stored in their original form. Calibration adjustments and adjustments to reduce data to standard conditions for comparability will be clearly documented, and raw data clearly distinguished from "corrected" data (i.e., data to which calibration and standardization adjustments have been applied).

Raw data and adjustments should be entered into a computer database and/or spreadsheet for correction, statistical analysis, manipulation, formatting, and summarizing to reduce the potential for human error.

D2 VALIDATION and VERIFICATION METHODS

Data reporting consists of communicating summarized data in a final form. QA for reporting consists of measures intended to avoid or detect human error and to correct identified errors. Such methods include specification of standard reporting formats and contents of measures to reduce data transcription errors. Study design and resulting data will undergo peer review by qualified reviewers capable of evaluating reasonableness of the data for the scientific design.

<u>Reports</u>: A report of all the summary study design characteristics, sample collections and analyses, data quality and results shall be presented by the analytical laboratories. Simple statistical tests of group treatment differences should be performed and presented as discussed above and will be conducted by EPA. All raw data and summary results of both data and summary statistics (means, standard deviations, ranges, etc.) should be tabulated by the laboratories. Study reports should be available within 60 days of receipt of acceptable laboratory results and reports.

QA records and project files will be maintained in accordance with standard project procedures. All QA records, logbooks, sample data forms, raw data summaries, and the like will be maintained until written directions for their disposal are provided.

D3 RECONCILIATION with DQOs

The project team will review any results which fall outside the DQOs and decide (per DURA 1992 and RAGS 1992) the extent of usability of results for the purposes of this investigation.

REFERENCES:

Casteel, S.W., Cowart, R.P. Weis, C.P., Henningsen, G.M. Hoffman, E. et al. (1997) Bioavailability of Lead in Soil from the Smuggler Mountain Site of Aspen Colorado (Accepted for Publication; *Fundimentals of Applied Toxicology*).

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EPA. 1999. Pilot-Scale Soil Characterization Study: Vasquez Boulevard and I-70 Site, Denver, Colorado. Prepared for USEPA Region 8. Prepared by ISSI Consulting Group, Inc. September 1999.

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Weis, C. P., Poppenga, R. H., Thacker, B. J., and Henningsen, G. M. (1994) Design of pharmacokinetic and bioavailability studies of lead in an immature swine model, LEAD IN PAINT, SOIL, AND DUST: HEALTH RISKS, EXPOSURE STUDIES, CONTROL MEASURES, MEASUREMENT METHODS, AND QUALITY ASSURANCE, ASTM STP 1226, M. E. Beard and S. A. Iske, Eds., American Society for Testing and Materials, Philadelphia, 19103-1187

Weis, C.P. and LaVelle, J.M. (1991) Characteristics to consider when choosing an animal model for the study of lead bioavailability. In: The Proceedings of the International Symposium on the Bioavailability and Dietary Uptake of Lead. Science and Technology Letters 3:113-119.

E. SOPs

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY
STANDARD OPERATING PROCEDURES

SOP	Title
0	General Procedures
1	Laboratory Equipment
2	Purchase of Supplies and Preparation of Reagents
3	Animal Purchase, Identification and Grouping
4	Animal Weighing, Feeding and Watering
5	Animal Health Evaluation
6	Collection and Submittal of Test Materials
7	Preparation and Administration of Doses
8	Data Recording and Reporting
9	Sample Labeling and Assignment of Sampling Numbers
10	Preparation of Samples for Analysis
11	Chain of Custody Forms and Procedures
12	Preparation and Analysis of Dose Verification Samples
13	Identification of Test Material
14	US EPA CLP Statement of Work for Inorganic Analysis
15	Methods for Metal Speciation
16	In Vitro Bioaccessability
17	Collection and Preparation of Urine Samples for Arsenic
18	Preparation and Analysis of Swine Urine for Arsenic

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 0

Title: General Procedures

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Steve W. Casteel 9/20/99

Study Director Date

SYNOPSIS: Outline of study general procedures.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	[signature]	9/17/99
Study QA Officer	W. Woldade / QAU	9-16-99
ISSI Consulting Group, Inc.	W J Brattin	9/16/99

GENERAL PROCEDURES

1.0 The study protocol along with the QAPP and its associated SOPs will be maintained in a Study Notebook. A copy of this notebook will be readily available to all team members.

2.0 All study operations will be in accord with these SOPs. Any deviations will be recorded.

3.0 All data will be electronically recorded or written in ink. Corrections to electronic data must be approved in writing by the Study Director. Corrections to written data must be accomplished by a single strike, the correct entry, a reason for the change unless obvious (e.g., spelling) and the initials of the person making the correction, accompanied by the date. The person making the correction should be the same as the individual who made the original entry.

4.0 Abbreviations used will be either defined at first use in each document or be defined in *Stedman's Medical Dictionary*.

5.0 Measurements will be made in metric units.

6.0 The Quality Assurance Officer (QAU) will maintain the Standard Operating Procedures (SOPs) and any revisions.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 1

Title: Laboratory Equipment

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Steven W. Casteel 9/20/99
Study Director Date

SYNOPSIS: All equipment, small instruments and laboratory items will be maintained, calibrated, and operated as described herein.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	[signature]	9/17/99
Study QA Officer	[signature] Goldade / QAU	9/16/99
ISSI Consulting Group, Inc.	WJ Brattin	9/17/99

LABORATORY EQUIPMENT

1.0 All equipment maintained and used as described in the provided manufacturer's manual. The manufacturers manual will be kept near each equipment item.

2.0 All equipment used to weigh or measure materials will be calibrated to ensure they are accurate, and these calibrations will be repeated periodically throughout the study, as detailed below. Log books documenting the calibration of scales, balances, and pipettes will be maintained throughout the course of the study.

a. Scales and balances used in the study will be calibrated no less frequently than once per week, using a set of calibration weights. All calibrations will be recorded in a log book.

b. Re-pipettors will be calibrated prior to processing each "batch" of samples. This will be done by weighing a standard volume of distilled water. Delivery of volumes outside the manufacturer specifications will be reported and corrected.

c. Small volume pipetting will be conducted with electronic digital pipettes of known accuracy and precision. Accuracy and precision will be checked using the method described in b (above) at the lowest and highest manufacturer's recommended volumes. In addition, a commercially available calibration kit will be used to assure pipette accuracy (Medical Laboratory Automation, Inc., Pleasantville, New York). The commercial kit will be used on a monthly basis or for each batch of samples to be processed, at the discretion of the Study Director.

d. Freezer and refrigerator temperatures will be "read" by a standard high-low thermometer left in the unit. The weekly high-low will be recorded.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 2

Title: Purchase of Supplies and Preparation of Reagents

Total Pages 3

APPROVALS:

ISSI Consulting Group, Inc.
Author

Steven W. Casteel 9/20/99

Study Director Date

SYNOPSIS: This SOP specifies methods for preparing laboratory reagents and for ensuring those reagents and disposable laboratory equipment items are not significantly contaminated with arsenic.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPhi	9/17/99
Study QA Officer	[signature] / QAU	9-16-99
ISSI Consulting Group, Inc.	W S Brattin	9/16/99

PURCHASE OF SUPPLIES AND PREPARATION OF REAGENTS

1.0 INTRODUCTION

Various disposable supplies and laboratory reagents are needed to perform the investigations that comprise this study. This SOP details the preparation of laboratory solutions and a protocol to follow to ensure that the supplies and reagents are not significantly contaminated with arsenic.

2.0 SUPPLIES AND EQUIPMENT

A variety of items are required to perform the investigations including:

- Feed containers
- Purple top Vacutainers® (10 mL and 3 mL)
- Blue top 15 mL Falcon® centrifuge tubes
- Whirlpac® plastic bags
- Micropipette tips
- Scintillation vials (5 mL)
- Plastic scissors
- Plastic forceps
- Teflon screw cap containers
- Volumetric pipettes
- Volumetric flask
- Coors crucible
- Agate mortar/pestle

Prior to employing such items in an investigation, one or more samples of each item or each batch, as appropriate, must be tested to ensure that the equipment will not contribute significant arsenic contamination.

3.0 STOCK REAGENTS

Purchase only the highest purity reagents available, with special attention to the level of arsenic contamination. Store all stock reagent bottles in a locked room. Never place any object into a stock reagent bottle. Rather, pour portions of the chemical into separate clean, arsenic-free containers, as needed. Never return any material to a stock reagent bottle.

4.0 STOCK SOLUTIONS

Prepare fresh stock solutions for each new study. Label each prepared solution with the following information:

- Reagent name
- Composition
- Date prepared
- Expiration date
- Initials of preparer

Store all stock solutions in a secure cabinet. If any visible sign of precipitation or microorganism growth is detected in stock solution, discard all of that stock solution and prepare fresh. Never place a pipette or any other object in a stock solution bottle. Rather, pour stock solution into a clean tube as needed. Never return unused solution to the stock bottle.

4.1 Sodium Arsenate Solutions

<u>Stock Solution A</u>

Weigh 41.6 grams of sodium arsenate ($Na_2HAsO_4 * 7H_2O$) and dissolve in distilled water to a final volume of 1 liter. (Volumes may be adjusted as necessary to prepare amount of reagent needed for study). The concentration of arsenic in this solution is 10 ug/uL.

<u>Stock Solution B and C</u>

Prepare Stock Solutions B and C by diluting Stock Solution A as follows:

> Stock Solution B = 20 mL of Stock Solution A + distilled water to a final volume of 100 mL (1:5 dilution). The concentration of arsenic in this solution is 2 ug/uL.
>
> Stock Solution C = 10 mL of Stock Solution A + distilled water to a final volume of 100 mL (1:10 dilution). The concentration of arsenic in this solution is 1 ug/uL.

<u>IV Solution</u>

This Stock Solution is used for intravenous dosage or preparation of arsenic standards. Prepare the IV Stock Solution by diluting Stock Solution B as follows:

> IV Solution = 1 mL of Stock Solution B + distilled water to a final volume of 100 mL. The concentration of arsenic in this solution is 0.02 ug/uL or 20 ug/mL.

4.2 Sodium Arsenate Check Standards

The following amounts of the IV Stock Solution will need to be diluted with control animals urine to 60 mL in a 100 mL graduated cylinder to achieve the required check standards:

Arsenic Low (10 ug As/L urine)

AsLow = 30 uL of IV Stock Solution + urine from control animals to a final volume of 60 mL. Mix well.

Arsenic Medium (30 ug As/L urine)

AsMed = 90 uL of IV Stock Solution + urine from control animals to a final volume of 60 mL. Mix well.

Arsenic High (100 ug As/L urine)

AsHigh = 300 uL of IV Stock Solution + urine from control animals to a final volume of 60 mL. Mix well.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 1) **SOP No.** # 3

Title: Animal Purchase, Identification and Grouping

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/20/99
Study Director Date

SYNOPSIS: Each animal purchased for potential use in an investigation must be assigned a permanent and unique identification number. All animals selected for inclusion in an investigation must be assigned to dose/treatment groups at random. This SOP describes how these requirements will be achieved.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	[signature]	9/17/99
Study QA Officer	W. Goldade / QAU	9.16.99
ISSI Consulting Group, Inc.	WJ Brattin	9/16/99

ANIMAL PURCHASE, IDENTIFICATION, AND
ASSIGNMENT TO GROUPS

1.0 ANIMAL PURCHASE

All animals used in these investigations will be purchased from the Pig Improvement Corporation (PIC) facility located near Monroe City, Missouri. All animals will be intact males of the genetically defined Line 26. The number of animals purchased should be approximately 10% more than the number called for in the study plan. The body weights and/or ages of the animals purchased should all be as uniform as possible. The target body weight at time of purchase is about 7-8 kg.

2.0 ANIMAL IDENTIFICATION

Immediately upon receipt of the animals, each animal will be given an ear tag which contains a permanent and unique identification number. This number will consist of the investigation number followed by a two-digit identification number. For example, animals in Investigation 3 would be assigned consecutive ID numbers beginning with 301.

3.0 PRE-INVESTIGATION HANDLING

The ear tag number of each animal will be entered into the logbook on the page designed for recording pre-investigation observations. Animals will be fed and watered as detailed elsewhere, and the body weight of each animal will be recorded prior to dosing during the holding period. Any animals that do not appear healthy or are not growing at the same rate as the other animals will be excluded from the investigation. Of the remaining animals, the heaviest and the lightest will be excluded in an alternate fashion until the number remaining is equal to the number called for in the study protocol. Each of these animals is then assigned to a dose/treatment group at random, as detailed below.

4.0 RANDOM ASSIGNMENT TO TREATMENT GROUPS

a. Prepare a list of the animals in ear tag order.

b. Use a computer to generate a series of random numbers, assigning these numbers in turn to each animal in the list.

c. Sort the animals sequentially based on the random numbers.

d. Assign the first four animals to group 1, the next 4 to group 2, etc.

e. Sort animals sequentially within assigned groups.

This randomization procedure is general and can be used to assign Sample Numbers or any other items that requires a random approach.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 4

Title: Animal Weighing, Feeding and Watering

Total Pages 4

APPROVALS:

ISSI Consulting Group, Inc.
Author

Steven W. Casteel 9/20/99
Study Director Date

SYNOPSIS: This SOP specifies the feeding and water requirements for all animals used in this study, and provides steps to ensure that neither the feed nor the water is an important source of exposure.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPhi	9/17/99
Study QA Officer	M. Goldade / QAU	9.16.99
ISSI Consulting Group, Inc.	WJ Brattin	9/16/99

ANIMAL WEIGHING, FEEDING AND WATERING

1.0 FEEDING

All investigations performed in this study call for animals to be provided with 100% of their daily food requirements. This is achieved by supplying each animal with food equivalent to 5% of its body weight each day. Since the animals are expected to grow significantly (0.3 to 0.8 kg/day) over the investigation period, the food portions must be constantly adjusted upward over time.

1.1 Food Supply

The feed used in these experiments will provide 100% of the recommended dietary requirements of swine. The feed will be analyzed prior to usage to confirm low arsenic concentrations. The dietary composition will be reviewed by a swine nutritionist. This feed will be purchased from Ziegler, Inc., and a detailed analysis of the composition will be provided with each lot purchased.

1.2 Weighing Schedule

Each animal must be weighed once every three days of the investigation, normally beginning on day -1. These weights will be used to calculate the appropriate amount of feed to give during the following three days (see below). Animals will also be weighed on the day of sacrifice. All body weights will be recorded in the laboratory log book to the nearest 0.1 kg.

1.3 Calculation of Food Portions

Food portions administered in these experiments will be based on the **mean body weight** (MBW) of all animals on study. Further, the mean body weight used will be adjusted to account for the gain in body weight expected to occur over the next two days following weighing, such that the mean body weight used is the estimated weight on day 2 of the 3-day period. This adjustment (based on the growth rates observed in EPA Phase I experiments) simply requires adding 1 kg to the mean body weight measured.

An example calculation is shown below.

Mean Body Weight (day -1) 8.3 kg (measure)

Estimated MBW on day +1 9.3 kg (MBW + 1 kg)

Calculation of food portion to be given twice each day on days 0, 1, and 2:

$$Portion\ (g) = (1/2)(0.05)(9.3\ kg)(1000\ \frac{g}{kg}) = 232.5\ g$$

Weigh feed portions (accurate to within ± 5% of the target) into disposable paper containers. The total number of portions needed is equal to six times the number of animals on paper study (two portions per day for each of three days). Provide one portion to each animal twice each day according to the time schedule specified in the experimental protocol.

Table 1 summarizes the growth rates observed during Phase I, and illustrates the range of food portions that may be required.

Feed Analysis

To ensure the feed delivered actually contains low arsenic levels, two random samples (each about 5 g) from each batch of food provided by the supplier will be placed in separate 15 mL Falcon® centrifuge tubes and sent to the laboratory for arsenic analysis prior to beginning each investigation.

2.0 WATERING

Water Supply

The protocol for all investigations performed during this study calls for animals to be provided with drinking water ad libidum. The source of the drinking water will be the municipal drinking water system, and drinking water will be provided to each cage via a pipe and nozzle which is activated by the animal. Laboratory technicians will check each day to ensure that all water delivery nozzles are functioning properly.

Water Analysis

To ensure the drinking water delivered to the animals is not significantly contaminated with arsenic, one sample (about 5-10 mL) will be drawn at random from a drinking water nozzle and placed in 15 mL Falcon® tube for shipment to the analytical laboratory for arsenic analysis (Detection limit = ~2 ug/L). This process will be repeated approximately once each week during the investigation.

TABLE 1 SUMMARY OF SWINE GROWTH CURVES OBSERVED DURING PHASE I

Study Day	Mean Body Weight (kg)	Food Portion (g) (twice/day)
-7	8.8	220
0	10.7	268
5	12.8	320
9	14.8	370
13	17.1	428
17	19.6	490
21	22.6	565
25	25.7	643

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 5

Title: Animal Health Evaluation

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author
Steven W. Casteel 9/20/99
Study Director Date

SYNOPSIS: Animal observations will be conducted by an attending veterinary clinician as described herein.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPh	9/17/99
Study QA Officer	W. Goldade	9-16-99
ISSI Consulting Group, Inc.	WJ Brattin	9/16/99

ANIMAL HEALTH EVALUATION

1.0 Swine chosen for each investigation will be monitored throughout the investigation to identify any evidence of disease. This monitoring program will consist of the following elements:

1.1 Daily observation by the PI or designated assistant, with consultation as needed by a board-certified food-animal clinician. Observations for each animal will be recorded daily on a health-status chart attached to the cage of each animal. Observations will be generally akin to the "SOAP" (subjective, objective, analysis, plan) process. If any interventive steps are taken for an animal (e.g., administration of antibiotics), this action shall also be recorded on the chart for that animal.

1.2 Any pig that dies during the study period will have a thorough post-mortem examination conducted to determine the cause of death. The post-mortem examination will include gross and histologic examinations and any ancillary tests, such as microbiology, deemed appropriate by the veterinary pathologist. All observations and findings will be recorded.

1.3 Medical records from the swine producer and the producer's veterinarian, including documentation of health status, will be available if needed to assess overall swine herd health, history of vaccinations, etc.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 6

Title: Collection, Preparation and Shipment of Test Materials

Total Pages 7

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/20/99
Study Director Date

SYNOPSIS: This protocol describes the collection and submission of specific test materials.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPh	9/17/99
Study QA Officer	W. Gaidade / QAU	9-16-99
ISSI Consulting Group, Inc.	WJ Brattin	9/16/99

COLLECTION, PREPARATION, AND SHIPMENT OF TEST MATERIALS

1.0 SAMPLE SELECTION

The primary reason for testing site-specific samples in this study is to obtain information that will improve the accuracy of exposure and risk calculations. Therefore, it is expected that site samples will be selected to be representative of materials which are of current or potential future human health concern. Samples may either be from discrete locations, or may be composites from an area. Samples may either be relatively pure mineral or physical forms, or may be mixtures that are typical of mixtures found on site. It is recommended that the input of all concerned parties be considered before final sample selection.

2.0 SAMPLE COLLECTION

2.1 Target Concentration

The concentration of arsenic in the material submitted must be high enough to provoke a measurable response in the animal test system (if the arsenic is bioavailable). The most convenient concentration is 200 to 2,000 ppm, but higher or lower concentrations are acceptable.

However, no sample less than 200 ppm should be submitted without first discussing and receiving approval from the EPA regional toxicologist.

2.2 Amount Required

The target amount of material required is about 1 kg. Higher amounts may be needed if the concentration value is near the low end of the acceptable concentration range. Contact the EPA regional toxicologist if it is not possible to obtain this much material for testing. It should be noted that the material collected must be large enough to provide a split to the PRPs, if requested.

3.0 SAMPLE COLLECTION AND PREPARATION

Samples will be collected according to the protocols developed for the VB-I70 Intensive Sampling QAPP.

4.0 SAMPLE LABELING

Each sample of test material must be labeled with the following information:

- Site name
- Sample description
- Sample collection date
- Initials of person collecting sample
- Arsenic concentration value (mg/kg)

6.0 SAMPLE SHIPPING

ISSI will send the sample under chain-of custody procedures to the Principle Investigator (PI). All packages will be opened by the PI or authorized staff following chain-of-custody procedures. Receipt of all samples or test chemicals will be recorded. Chain-of-custody forms will remain attached to all test samples.

7.0 Storage of Test Materials.

All test samples will be retained in their original shipment containers and stored in a secure room that is locked at all times except when it is being used for preparation of doses or samples, or other operations associated with performance of these investigations. Access to the locked room will be restricted to the PI or dedicated staff authorized by the PI.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 2) **SOP No.** # 7

Title: Preparation and Administration of Doses

Total Pages 5

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel

Study Director Date

SYNOPSIS: Dosing of animals will be carried out as described in this SOP.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPhi	9/17/99
Study QA Officer	M. Goldade / QAU	9.16.99
ISSI Consulting Group, Inc.	WJ Brattin	9/16/99

PREPARATION AND ADMINISTRATION OF DOSES

1.0 INTRODUCTION

Bioavailability studies call for exposure of animals to known doses of sodium arsenate and to arsenic in various samples of soil or mine waste. All doses will be based on the mean body weight of the animals on study, using a three-day weighing cycle (see SOP 4).

2.0 ORAL EXPOSURE

2.1 Sodium Arsenate

Oral exposure to sodium arsenate is achieved by placing a small volume of sodium arsenate stock solution into a depression in a 5 g mass of moistened feed ("doughball"). After the stock solution has permeated into the doughball and no free liquid remains, the depression is filled by squeezing the dough ball in on itself, and the doughball is administered to the animal by hand feeding.

All animals in each dose group will receive the same volume of sodium arsenate stock solution, based on the mean body weight of all animals in the group. (The precise dose to each animal will subsequently be calculated from the individual measured body weights). Calculate the volume of stock solution to place in the dough balls of each dose group (twice each day) using the following equation:

$$Vol = 0.5 \left(\frac{MBW \cdot Dose}{Conc} \right)$$

where:

Vol = Volume of stock solution (uL)
MBW = Mean body weight (kg), adjusted as detailed in SOP 4
Dose = Target dose for the group (ug/kg-d)
Conc = Concentration of stock solution (ug/uL)

Three different stock solutions of sodium arsenate will be used as described in SOP 2. Choose Stock Solution A (10 ug/uL), Stock Solution B (2 ug/uL) or Stock Solution C (1 ug/uL) so that the volume of liquid added to the dough ball is at least 20 uL and not more than 100 uL.

EXAMPLE CALCULATIONS

	Example 1	Example 2	Example 3
Mean Body Weight (kg)	9.7	14.3	15.8
Target Dose (ug/kg)	25	50	125
Volume of A (uL)	12.1	35.8	98.8
Volume of B (uL)	60.6	178.8	493.8
Volume of C (uL)	121.3	357.5	987.6
Solution Selected	B (60.6 uL)	A (35.8 uL)	A (98.8 uL)

All volumes must be measured with an accurate adjustable micropipette using disposable plastic tips.

2.2 Soil Samples

Administration in "Doughballs"

Oral exposure to arsenic in test soil or mine waste is achieved by placing the required mass of the test material into a depression in a mass of moistened feed ("doughball"). The size of this doughball should be approximately 5 g ($\pm$ 1 g). The depression is then filled by squeezing the doughball in on itself, trapping the test material in the center. Typically, all of the required mass of soil for each dose can be placed into a singe doughball. If the mass of soil required is too large to encapsulate into a single doughball, the mass of soil shall be divided into approximately equal portions and placed in the minimum number of doughballs reqired to contain the soil.

Calculation of Soil Mass

All animals in each dose group will receive the same mass of test material, based on the mean body weight of all animals within the dose group. (The precise dose to each animal will subsequently be calculated from the individual measured body weights). Calculate the mass of test material to administer to each dose group (twice each day) using the following equation:

$$Mass = 1/2 \left(\frac{MBW \cdot Dose}{Conc} \right) (1000\ \mu g/mg)$$

where:

Mass = Mass of test material (mg)
MBW = Mean body weight (kg)
Dose = Target dose for the group (ug/kg-d)
Conc = Concentration of arsenic in the test material (ug/g or ppm)

A few example calculations are shown below. The calculations for each group on each day must be recorded.

	Example 1	Example 2	Example 3
Mean Body Weight (kg)	9.7	14.3	15.8
Target Dose (ug/kg)	25	50	125
Concentration As in soil (ppm)	500	1000	3000
Soil mass per dose (mg)	242.5	357.5	329.7

Soil doses must be weighed with a precision of at least ± 5%.

2.3 Soil Mixing Prior to Weighing

It is expected that the bulk soil sample will be non-homogeneous with respect to particle size, and the concentration and form of arsenic is expected to vary as a function of particle size. Therefore, it is important that the soil be well-mixed prior to removal of the dose aliquots. This is achieved by placing the bottle containing the bulk soil sample on a roller operating at low speed for about 30 minutes. After rolling, the bottle should be further mixed by inverting five times. It is important that vigorous methods of mixing not be used, since this might lead to a redistribution of the particle size distribution.

2.4 Oral Dose Verification Samples

At least two extra dough balls (or sets of doughballs, if more than one doughball is required to administer the soil) should be prepared for each dose "batch" (a "batch" is a group of doughballs, sufficient for three days administration to a particular set of animals). After all doughballs in the batch are prepared, select two at random, wrap them (individually) in plastic wrap, and place both in a Whirl-Pac plastic bag labelled with the appropriate "self-reading" sample identification number, as described in Section 1.2 of SOP 9. ISSI will provide preprinted self-reading sample labels to UM-C for labeling these storage bags. The adhesive sample label should be affixed to the plastic bag and covered with clear packaging tape to avoid loss of the labels during storage. [NOTE -- If it is determined that the tape/label method does not work, assigned sample labels will be written on the plastic bags with an indelible ink marker.] Store (archive) all potential dose verification samples in the freezer until the end of the study.

At the end of the study, EPA and the PI will decide what percentage of all dose verification samples to send to the laboratory for analysis. This will always be at least 5%, but may be higher. After the percent of samples to be analyzed is specified, ISSI will select (at random) the specific samples to be tested, and provide a list of these samples along with a full set of sample tags, a "sample detail" sheet (this shows which dough balls receive which tags), and a chain-of-custody sheet. The archived bags containing the specified samples should be located, and one of the doughballs within the bag removed and placed in a separate bag (labeled with the assigned sample tag number) for shipment to the laboratory. Replace the remaining dough ball sample in the storage box in the freezer as soon as possible.

After all samples have been placed in bags as above, check the COC form, noting any discrepancies in the "Comments" field. Ship the frozen dough balls (on ice/blue ice) to the analytical laboratory for sample preparation and analysis as detailed in SOP 12.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 1) **SOP No.** # 8

Title: Data Recording and Reporting

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Steven W. Casteel 9/26/99

Study Director Date

SYNOPSIS: Data recording and reporting will be conducted according to these procedures.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	[signature]	9/17/99
Study QA Officer	M. Woldade / QAU	9.16.99
ISSI Consulting Group, Inc.	W J Brattin	9/16/99

DATA RECORDING AND REPORTING

1.0 INTRODUCTION

Data that must be recorded and reported for each animal over the course of each study includes:

- Measured body weights
- Food supplied
- Doses administered
- Samples collected
- Exceptions or variances from the planned protocol

For each investigation performed during these experiments, a log book will be provided for entry of these data items on the schedule required.

2.0 DAILY LOG RECORDING PROCEDURE

There will be one log book for each investigation, and this log book will contain one or more pages per day (as needed), beginning on the day the animals arrive at the facility. Each page will list the animals on test down the left side, and will provide space to record any data items scheduled for collection on that day. On days when biological samples are scheduled for collection, the logbook will specify the sample identification number to be attached to each sample after collection.

All data must be recorded daily on the appropriate page of the log book. All data must be recorded at the time each value is measured or observed. All entries must be in ink (preferably black). Take care to ensure entries are readily legible. In the event that a data entry error is made, the incorrect entry must be crossed out with a single line through the value and the correct value entered in an adjacent location. This change must be initialed and dated by the person making the change. If another person is present, have that person initial the change as well. An explanatory note giving a brief reason for the change must also be provided. The log book should also be used to record any problems, errors, exceptions or variances from the intended protocol, along with any explanatory notes or other comments.

3.0 DATA REVIEW AND SUBMITTAL

At the completion of data entry for a given day, the log book will be provided to the principle investigator for review and signature. A copy of each page of the daily log will be sent to ISSI no less frequently than once per week. Typically, transmittal of the daily log pages will occur every Tuesday, at the same time that samples are transmitted to the analytical laboratory.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 1) **SOP No.** # 9

Title: Sample Labeling and Assignment of Sample Numbers

Total Pages 4

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/26/99
Study Director Date

SYNOPSIS: This SOP describes a system for assigning labels and sample numbers to each sample so that each may be unambiguously identified.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPW	9/17/99
Study QA Officer	M. Woldade / QAU	9.16.99
ISSI Consulting Group, Inc.	W J Brattin	9/16/99

SAMPLE LABELING AND ASSIGNMENT OF SAMPLE NUMBERS

1.0 DECIPHERABLE LABELS

All samples collected during this study will be assigned a unique label that can be readily deciphered. The nomenclature for labels assigned to biological samples and dose verification samples are detailed below.

1.1 Biological Samples

Each biological sample (blood, tissue, bone) label will have 5 elements, as follows:

PHASE. All labels will begin with a two letter code to indicate from which study the sample is derived (e.g., XX-).

INVESTIGATION NUMBER. The next element of the label will be the investigation number for a given phase. Investigations will be numbered sequentially using Arabic numerals. The number of each investigation will also be clearly indicated in the study protocol.

ANIMAL NUMBER. The next element of each label is the unique identification number assigned to each animal at the start of each investigation (see SOP 3).

TREATMENT DAY. The next element of the label is the day of the investigation on which the sample was collected. Day zero is the first day of dosing/treatment. Samples collected before dosing should be identified with the label "-" (e.g., -7 means 7 days before exposure/treatment begins).

SAMPLE TYPE. The final element of the sample label is the sample type, using the following codes: U = urine and P = feces. Other codes can be used as needed if other sample types are prepared or collected.

Thus, a sample labeled "XX-3 317-4-U" would be a sample of urine collected from animal 317 on the 4th day of investigation 3 in Phase XX. Likewise, a sample labeled "XX-2 283-15-P" would be a sample of feces collected from animal 283 on the 15th day of investigation 2 in Phase XX.

1.2 Dose Verification Samples

Two extra dough balls will be prepared for each three-day batch for each dose group for possible use as dose verification samples. Both extra doughballs from each group should be placed in individual plastic bags, and assigned labels using a code system similar to that above. However, substitute the group/treatment number for the animal number. Also, since the doughballs are prepared in batches adequate for three days, give the range of days rather than a discrete day. Thus, a bag labeled "XX-4 Grp5-6to8-DV1" would identify the first dose verification sample for Group 5 on days 6, 7, and 8 of Investigation 4 in Phase XX.

2.0 SAMPLE IDENTIFICATION NUMBERS

Analysis of all samples sent to the laboratory is blind, except for identification of the sample matrix. Therefore, each sample is assigned a non-decipherable sample number. These numbers will be provided by ISSI and preassigned to samples as shown in the log book. As an added level of protection against error, the archive sample (e.g. whole blood, tissue sample) that is used to prepare a sample for analysis will be labeled with the same sample number that is assigned to the prepared sample, except the suffix "-AS" ("archive sample") will be included.

3.0 SELF-ADHESIVE LABELS

As noted above, all sample labels and sample numbers will be pre-assigned and listed in the laboratory notebook. To reduce effort and avoid transcription errors, ISSI will prepare sheets of pre-coded self-adhesive labels for attachment to all collection and storage containers and all QA samples sent to the laboratory for analysis. A hypothetical example of such a label sheet is shown in Figure 1. As shown, the labels are arranged from top to bottom in order of cage number. This is because sample collection will proceed in this order.

FIGURE 1
EXAMPLE LABEL SHEET

US EPA REGION 8 SWINE BIOAVAILABILITY STUDY -- PHASE XX
INVESTIGATION 3, DAY 5

Cage No	Pig No.	Sample Label	Sample Number		
			Archive	ESD	Splits
1	337	XX-3 337-5-U	8-930567-AS	8-930567	--
2	318	XX-3 318-5-U	8-930594-AS	8-930594	8-930567CDC 8-930567PRP
3	355	XX-3 355-5-U	8-930561-AS	8-930561	--
4	314	XX-3 314-5-U	8-930577-AS	8-930577	--
5	311	XX-3 311-5-U	8-930582-AS	8-930582	8-930582CDC 8-930582PRP
6	305	XX-3 305-5-U	8-930575-AS	8-930575	--
etc					
etc					

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 1) **SOP No.** # 10

Title: Preparation of Samples for Analysis

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/20/99

Study Director Date

SYNOPSIS: Samples of water and feed will be prepared for analysis according to the procedures described herein.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPW	9/17/99
Study QA Officer	J. Woldade / QAU	9.16.99
ISSI Consulting Group, Inc.	W S Brattin	9/16/99

PREPARATION OF SAMPLES FOR ANALYSIS

Samples of water and feed will be prepared for shipment to the analytical laboratory as follows.

1. Water:

   a. Random samples of drinking water (approximately one per week) will be placed in 15 mL Falcon® tubes and shipped unprocessed to the analytical laboratory.

   b. Random samples (approximately one per week) of the double distilled water used to prepare samples and reagents will be placed in 15 mL Falcon® tubes and shipped unprocessed to the analytical laboratory.

2. Feed:

   a. 0.50 gram sub-samples of feed will be placed into a Teflon container and 5 ml of 70% nitric acid added. The digest will be brought to a 25 mL volume with double distilled water.

   b. Two gram portions of feed not utilized for analysis will be stored at ca. -10°C for potential future reanalysis.

3. Dose Verification Samples:

   As described in SOP 7, two extra "doughballs" will be prepared for each dose group for each three-day dosing period. Doughballs selected for analysis will be prepared and analyzed as detailed in SOP 12.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 1) **SOP No.** # 11

Title: Chain of Custody Forms and Procedures

Total Pages 6

APPROVALS:

ISSI Consulting Group, Inc.
Author

Steven W. Casteel 9/20/99
Study Director Date

SYNOPSIS: This SOP describes a system for maintaining a chain of custody record for all samples generated.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPlin	9/17/99
Study QA Officer	M. Goldade / QAU	9-16-99
ISSI Consulting Group, Inc.	W J Brattin	9/16/99

CHAIN OF CUSTODY FORMS AND PROCEDURES

All samples collected during this study must be accounted for and traceable from the time of collection through analysis. A chain of custody (COC) form is created at the time that samples are originally collected, and this form must accompany the samples during each step of the preparation and analysis sequence.

1.0 Custody of Samples at the Animal Testing Facility

Collection of Primary Samples

Each day that a group of samples is collected, a chain of custody form should be filled out to accompany those samples. These forms will be pre-printed prior to each investigation, based on the detailed protocol for that investigation. Each form will contain a unique identification number. These forms contain the following information:

- The Phase and Investigation Number
- A list of the sample numbers on all samples collected
- The date each sample was collected
- The type of sample (blood, liver, kidney, bone, water, etc.)

An example form is shown in Figure 1. The person responsible for collecting the samples should carefully review the pre-printed sheet to ensure that the samples collected match the list on the COC form. Any incorrect entries should be changed by drawing a single line through the entry and entering the correct information adjacent. All entries and changes must be made in ink, dated, and initialed. When all entries are correct, the sheet should be signed and dated.

Transfer to the Laboratory Technician

Most primary samples (blood, tissue, bone) require preparation before being sent to the analytical laboratory. When the samples are transferred from the person who collected the samples to the person responsible for preparing the samples, this transfer should be recorded on the COC sheet. The technician who receives the samples is responsible for checking to ensure that all samples on the COC form are actually provided and are in good condition. Any exceptions should be noted on the form.

Security of Primary and Prepared Samples

The PI is responsible for ensuring that all primary and analytical samples generated at the animal facility are maintained in a secure location and that no one has access to the samples except the PI or staff authorized by the PI.

2.0 Transfer of Samples from the Animal Facility to Other Locations

Whenever samples are sent from the animal facility to the analytical laboratory or any other location, a careful record of this transfer must be kept. The person who sends the package is responsible for ensuring that the contents of the package and the COC forms are in agreement. All samples and the accompanying COC forms must be securely enclosed in a shipping container, and this container must be sealed with an EPA custody seal. The EPA custody seal should be over-wrapped with clean packing tape to ensure the seal is not broken accidentally during shipment. A copy of all COC forms sent to offsite locations is maintained by the facility.

Whenever a shipment of samples is being sent to the analytical laboratory, the person sending the package should call ahead to notify the contract Laboratory of the time which samples will be shipped and the expected arrival date.

3.0 Receipt and Custody of Samples at the Analytical Laboratory

Responsibilities

The analytical laboratory will have a designated Sample Custodian who is responsible for insuring compliance with the provisions of this SOP. The lab will also designate an Alternate Sample Custodian. Any laboratory employee may receive samples, provided they follow the provisions of this SOP.

Sample Receipt

Samples are received from two main sources. These are direct delivery (hand carried) from the sampling team or delivered by a third party carrier (e.g. Federal Express).

> For hand carried samples, the sampling team member will deliver the samples and chain-of-custody documents to the receiving lab employee. The sampler will remain during the opening and inspection process.
>
> When third party delivery occurs, the shipping container is received and secured until opening. It is customary for the shipper to require a signed receipt form. A copy of this receipt is included in the custody record which becomes part of the final data package.

Opening the shipping container and inspecting the contents.

WARNING: It is possible for sample spillage, leaking containers or sample adhering to containers to pose health problems. The receiver must determine the proper level of personal protection required. A lab coat, gloves and goggles represent a minimum protective level for all persons present. Additional protection may include the use of a respirator or fume hood while opening and inspecting the shipment. The room selected for sample receipt must contain a fume hood and allow for easy containment and clean-up in case of spills.

Before opening the shipping container, it should be inspected for signs of damage. Note the condition of custody seals and open the shipping container. If third party shipping was used, the chain-of-custody (COC) forms should be located inside the container. The condition of the container and custody seals are now noted on the COC forms.

Remove each container in the shipment and check for damage, spillage or leakage. Spillage from a broken or leaking sample should be treated with an appropriate absorbent. Dispose of the absorbent and any broken containers according to Hazardous Waste Management procedures.

Cross-reference the sample tag with the sample identification on the COC forms. Any discrepancies including missing or mis-labeled samples, spillage or broken containers should be noted on the COC. It may be possible to identify samples with missing or illegible tags through some other characteristic. This should be carefully recorded on a separate document. This document will become part of the case narrative which accompanies the final report. Any discrepancies should also be verbally reported to the site project officer. Samples which can not be uniquely identified will not be analyzed, unless so directed by the analytical lab manager.

Samples are grouped by type of matrix and placed in secondary containers appropriate to the sample size and type. Each sample is checked for accuracy of its sample tag/label vs. entries on the COC form. The date and time of receipt are entered in the proper boxes of the COC form. The receiver then signs the box "Received at Laboratory by:". Any additional comments are entered at this time in the comments section of the form.

Sample Storage

In some cases, the sample receiver may distribute the required samples (or fractions) to the appropriate analytical section for immediate analysis. In all other cases, the samples are segregated by parameter and locked in cold storage.

Controlled sample access is required during their storage period. Samples are deemed to be in custody during their residence at the laboratory.

Sample log-in and assignment of analyses

After securing the samples, the sample receiver gives the chain-of-custody forms, Lab Service Requests and sampler's notes to the Laboratory Information Management System (LIMS) manager. He/she then enters relevant sample information into the LIMS. The manager then makes copies of the COC forms for each analytical group involved and highlights the parameters relevant to each group. These copies are then distributed to the analysts. The originals go into the site project file.

Sample analysis

When the analyst is ready to begin sample processing, he or she obtains the appropriate key from the sample custodian. After removal of samples, the cooler is immediately locked and the key returned to the sample custodian. After sample extraction or analysis, unused portions are returned to the the locked refrigerated storage area.

Sample disposal

Sample remainders are kept in the locked refrigerated storage for a minimum of three years after the final report is sent to the data user and approved by the Quality Assurance Office (QAU). At any time after this three years, the Sample Custodian in consultation with the data user may designate the samples for disposal.

Transmittal of documents

When analyses are complete, the analytical results, letter of transmittal, chain-of-custody forms, LSRs and samplers notes will be sent to the data user. Copies of these documents and laboratory raw data will be kept in laboratory files for at least ten years.

FIGURE 1
CHAIN OF CUSTODY FORM

COC XXX

US EPA REGION 8 BIOAVAILABILITY STUDY PHASE XX
Investigation 3, Day 7

Samples Collected By: ______________________________
Signature Date

Index	Sample No.	Date	Matrix	Analytes	Remarks
1	8-931561	9/10/99	Urine	Arsenic	
2	8-931562	9/10/99	Urine	Arsenic	
3	8-931563	9/10/99	Urine	Arsenic	
4	8-931564	9/10/99	Urine	Arsenic	
5	8-931565	9/10/99	Urine	Arsenic	
6	8-931566	9/10/99	Urine	Arsenic	
7	8-931567	9/10/99	Urine	Arsenic	
8	8-931568	9/10/99	Urine	Arsenic	
9	8-931569	9/10/99	Urine	Arsenic	
10	8-931570	9/10/99	Urine	Arsenic	
11	8-931571	9/10/99	Urine	Arsenic	
12	8-931572	9/10/99	Urine	Arsenic	
13	8-931573	9/10/99	Urine	Arsenic	
14	8-931574	9/10/99	Urine	Arsenic	
15	8-931575	9/10/99	Urine	Arsenic	
16	8-931576	9/10/99	Urine	Arsenic	
17	8-931577	9/10/99	Urine	Arsenic	
18	8-931578	9/10/99	Urine	Arsenic	
19	8-931579	9/10/99	Urine	Arsenic	
20	8-931580	9/10/99	Urine	Arsenic	
21	8-931581	9/10/99	Urine	Arsenic	
22	8-931582	9/10/99	Urine	Arsenic	
23	8-931583	9/10/99	Urine	Arsenic	
24	8-931584	9/10/99	Urine	Arsenic	
25	8-931585	9/10/99	Urine	Arsenic	

TRANSFER OF CUSTODY RECORD

Transfer	Relinquished by: Signature	Relinquished by: Date/Time	Received by: Signature	Received by: Date/Time
1				
2				
3				
4				

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** # 12

Title: Preparation and Analysis of Dose Verification Samples

Total Pages 5

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/26/99
Study Director Date

SYNOPSIS: This SOP describes the method to be used for digesting and analyzing selected samples of dosing materials ("doughballs") administered to swine during these investigations. The results of these analyses are used to ensure that dose materials administered did contain the amount of arsenic intended.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	[signature]	9/17/99
Study QA Officer	[signature]	9-16-99
ISSI Consulting Group, Inc.	W J Brattin	9/16/99

PREPARATION AND ANALYSIS OF DOSE VERIFICATION SAMPLES

1.0 INTRODUCTION

This SOP provides a detailed description of the methods to be used to digest and analyze dose verification samples (approximately 5 g portions of feed mixed with various amounts of either sodium arsenate or test material) from these investigations. The SOP addresses different sample preparation techniques that may be necessary to provide complete dissolution of the arsenic species present in materials added to the feed portions. This SOP is written as a guideline for an experienced chemist. Many routine details or techniques of sample preparation are not reiterated in this document. Minor method changes may be dictated by sample-type variations and should only be made by experienced laboratory personnel.

2.0 SAMPLE HANDLING AND PRESERVATION

Samples will be received as frozen "doughballs" contained in a plastic bag. Prior to preparation, samples should be kept in their shipping containers to maintain integrity. Samples should be kept frozen until analysis to avoid possible loss of analyte by sample flow.

3.0 APPARATUS AND MATERIALS

3.1 Equipment

The following equipment items are required for sample preparation:

- Muffle furnace, capable of maintaining temperatures in the range of 400 - 500 degrees Centigrade.
- Ceramic crucibles.
- Erlenmeyer flasks, 125 or 250 mL, with watch glasses or small funnels for covers.
- Reagent dispensers or pipettes to deliver reagents at volumes and accuracies discussed below.
- Hotplate with variable temperature controller.
- Miscellaneous laboratory materials and equipment including volumetric glassware, sample digestate containers, water wash bottles, reagent grade water source, and protective clothing and paraphernalia.

3.2 Reagents

The following reagents are required for sample preparation:

- Nitric Acid (HNO_3), concentrated (c. 60%).
- Hydrogen peroxide (H_2O_2), concentrated (c. 30%).
- Reagent grade water (DI water), to meet or exceed specified purity.
- Stock solutions of known, certified elemental concentrations for sample spike preparation.
- High purity cellulose fiber (filter and ashing aid material).

4.0 HANDLING AND DIGESTION PROCEDURES

4.1 Sample Transfer

Each swine feed portion ("doughball") is a unit sample. The entire sample must be digested to ensure complete metals recoveries. If the sample has been allowed to thaw during shipping or storage, care must be taken to ensure that all phases of the sample are quantitatively transferred to the digestion flask. Oil and moisture separations have been noted in thawed samples.

4.2 Sample Digestion

The method outlined below should yield a digestate amenable to GFAA or ICP analyses of arsenic and other common metals of environmental interest. Two digestion schemes are provided. One is followed for samples spiked with soluble arsenic salts and the other for samples which contain added soil or soil-like components. Some variations of reagent quantities, digestion times, and final dilution volumes may be required to produce a suitable analytical matrix. It is suggested that preliminary digestion trials of anticipated sample types be performed prior to actual analyses to evaluate the applicability of the general method.

1. About 20 mL of de-ionized (DI) water is added to the transferred and thawed sample in a 250 mL erlenmeyer flask. Twenty-five mL of concentrated nitric acid is added and the slurried sample is allowed to stand several hours or over-night at room temperature.

2. With a small glass funnel (or small watch-glass cover) in the neck of the flask, to provide reflux action, the sample is heated on a hotplate until the majority of the organic matrix is disrupted and digested. The digestate should have a pale yellow to clear appearance at that point.

3. The funnel is removed to allow the aqueous nitric acid phase to reduce to about 10 mL. The flask is removed from the hotplate and cooled.

4. Two mL of 30% hydrogen peroxide is added and the flask is returned to the hotplate to initiate the oxidation step. Additional aliquots of peroxide are added (up to 30 mL) as needed to complete the destruction of organic material.

5. After cooling, about 20 mL of DI water and 5 - 10 mL of nitric acid are added to each flask and the contents are heated to near boiling.

At this point, samples containing soil or other soil-like test material should be set aside and handled as described in steps 6A - 8A.

6. The cooled digestate is diluted to a suitable volume for analysis. A minimum volume of 500 mL is suggested to avoid potential solubility problems.

7. Filtration of the finished digestate prior to GFAA or ICP analysis is suggested.

If the sample contains soil or soil-like material (slag, waste rock, etc), follow steps 6A to 8A, below.

6A. The soil-spiked sample digestates from step 5 (above) are filtered through Whatman 40 (or other ashless equivalent) filter papers. The filtrate and water wash aliquots are diluted to a suitable final volume. The filter paper and residue is ashed in a porcelain crucible at a final maximum temperature of 450 degrees centigrade. Cellulose filter-aid is recommended to facilitate this step.

7A. The ash residue is transferred to a digestion flask. Five mL of water and 5 mL of concentrated nitric acid is added to the sample. The sample is heated on a hotplate to reduce the volume to 3 - 5 mL. Five mL of water and two mL of hydrogen peroxide are added to the cooled sample. The treated samples are returned to the hotplate to initiate the peroxide reaction. Subsequent portions of peroxide may be added if required.

8A. The cooled samples are diluted to final volume for analysis.

5.0 QUALITY CONTROL

Contamination from handling, glassware, or reagents is monitored by the examination of a DI water blank sample digested in the same fashion as the samples. As the entire sample is consumed during sample preparation, no duplicate or matrix spike samples are possible.

6.0 DOCUMENTATION AND DATA HANDLING

Swine feed samples will be identified throughout the preparation and analysis steps by the sample number.

The concentration results of analysis will be converted to total micrograms of arsenic per sample. In the case of soil-bearing samples, the reported value will be the sum of the masses of arsenic measured from each digestate part.

7.0 REFERENCES

Graphite Furnace AAS, A Source Book, Walter Slavin, 1984, Perkin-Elmer Corp., Norwalk CT 06856

Standard Operating Procedure: Perkin Elmer 5100 Graphite Furnace Atomic Absorption Spectrophotometer, ESAT SOP AI 02, 09/92.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** #13

Title: Identification of Test Material

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/20/99
Study Director Date

Stan W. Casteel

SYNOPSIS: This SOP outlines steps to prevent confusion as to which test materials to administer as dose material in a specified study.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPW	9/17/99
Study QA Officer	M Woodade	9.16.99
ISSI Consulting Group, Inc.	W S Brattin	9/16/99

In order to eliminate any confusion which might result pertaining to the proper dosing material for a specific experiment, the following procedure will be implemented:

1. Only samples for use in the next experiment will be shipped to the University of Missouri.

2. Sample locations (descriptions) will be placed on dosing material containers, in large, bold letters.

3. Dosing material chain-of-custody tag numbers will be included in the transmittal letter in conjunction with the relevant sample description.

4. All chain-of-custody forms will be initialled by ISSI personnel prior to shipment.

5. Only dosing materials for the particular experiment will be accessible in the Missouri dose preparation laboratory.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** #14

Title: US EPA Contract Laboratory Program
Statement of Work for Inorganic Analysis

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/20/99
Study Director Date

SYNOPSIS: Method of Analyses Outline

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPlin	9/17/99
Study QA Officer	J. Goldade	9-16-99
ISSI Consulting Group, Inc.	WJ Brattin	9/16/99

Metals analysis will be performed in accord with appropriate EPA SW-846 methods.

SOP # 15 STANDARD OPERATING PROCEDURE

September 1999

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** #15

Title: Methods for Metal Speciation (ISSI SOP No. ISSI-VBI70-09)

Total Pages 17

ISSI Consulting Group, Inc.
Author

SYNOPSIS: Method of Analyses Outline

Date: September 3, 1999 (Rev. # 0) SOP No. ISSI-VBI70-09

Title: METAL SPECIATION AND QUANTIFICATION OF PERLITE

APPROVALS:

Author: ISSI Consulting Group, Inc. Date:

SYNOPSIS: A standardized method for speciating metals and perlite particles in solid samples is described. Equipment operating conditions, sample preparation and handling, and statistical equations for data analysis and presentation are included.

REVIEWS:

TEAM MEMBER	**SIGNATURE/TITLE**	**DATE**
USEPA Region 8	Bonita Lavelle /RPM	9/10/99
ISSI Consulting Group, Inc.	WJ Brattin	9/13/99

1.0 OBJECTIVES

The objectives of this Standard Operating Procedure (SOP) are to specify the proper methodologies and protocols to be used during metal speciation of various solid samples (including tailings, slags, sediments, dross, bag house dusts, and paint), residential soils and dusts for metals. The metal speciation data generated from this SOP may be used to assess the solid samples as each phase relates to risk. Parameters to be characterized during the speciation analyses include particle size, associations, stoichiometry, frequency of occurrence of metal-bearing forms and relative mass of metal-bearing forms. In addition, aliquots of solid samples can be analyzed separately for perlite, using the same methodology. Perlite particles are counted and sized based on the mineral constituents of each particle. This electron microprobe (EMP) technique, instrument operation protocols and sample preparation to be used during implementation of the Metals Speciation SOP are discussed in the following sections.

2.0 BACKGROUND

To date, numerous metal-bearing forms of soils have been identified from various environments within western mining districts (Table 2-1) (Emmons et al., 1927; Drexler, 1991 per. comm.; Drexler, 1992; Davis et al., 1993; Ruby et al., 1994; CDM, 1994; WESTON, 1995). This listing does not preclude the identification of other metal-bearing forms, but only serves as an initial point of reference. Many of these forms are minerals with varying metal concentrations (e.g., lead phosphate, iron-lead oxide, and slag). Since limited thermodynamic information is available for many of these phases and equilibrium conditions are rarely found in soil environments, the identity of the mineral class (e.g., lead phosphate) will be sufficient and exact stoichiometry is not necessary.

It may be important to know the particle-size distribution of metal-bearing forms in order to assess potential risk. It is believed that particles less than 250 microns (μm) are most available for human ingestion and/or inhalation (Bornschein, et al., 1987). For this study, the largest dimension of any one metal-bearing form will be measured and the frequency of occurrence weighted by that dimension. Although not routinely performed, particle area can be determined. It has been shown (CDM, 1994) that data collected on particle area produces similar results. These measurements add a considerable amount of time to the procedure and limit the total number of particles or samples that can be observed in a study.

Mineral association may have profound effects on the ability for solubilization. For example, if a lead-bearing form in one sample is predominantly found within quartz grains while in another sample it is free in the sample matrix, the two samples are likely to pose significantly different risk levels to human health. Therefore, associations of concern include the following:

1) free or liberated
2) inclusions within a second phase
3) cementing
4) alteration rims

3.0 SAMPLE SELECTION

Samples should be selected and handled according to the procedure described in the Project Plan.

4.0 SCHEDULE

A schedule for completion of projects performed under this Metals Speciation SOP will be provided in writing or verbally to the contractor along with monthly reporting requirements if large projects are performed. These schedules are based on an aggressive analytical program designed to ensure that the metals speciation analyses are completed in a timely period. Monthly reports are expected to reflect schedule status.

5.0 INSTRUMENTATION

Speciation analyses will be conducted at the Laboratory for Environmental and Geological Studies (LEGS) at the University of Colorado, Boulder or other comparable facilities. Primary equipment used for this work will include:

Electron Microprobe (JEOL 8600) equipped with four wavelength spectrometers, energy dispersive spectrometer (EDS), BEI detector and the TN-5600 data processing system. RJ Lee ZEPPELIN and DATALINK hardware may be used for image storage and processing. An LEDC spectrometer crystal for carbon and LDE-1 crystal for oxygen analyses will be used.

6.0 PRECISION AND ACCURACY

The precision of the EMP speciation will be evaluated based on sample duplicates analyzed at a frequency of 10%. The accuracy of the analyses will be estimated based on a number of methods, depending on the source of the data. Data generated by the "EMP point count" will be evaluated statistically based on the methods of Mosimann (1965) at the 95% confidence level on the frequency data following Equation 1.

$$E_{0.95} = 2P(100-P)/N \qquad \text{(Eq. 1)}$$

Where:

$E_{0.95}$ = Probable error at the 95% confidence level

P = Percentage of N of an individual metal-bearing phase based on percent length frequency

N = Total number of metal-bearing grains counted

For arsenic, the goal is to count 200 particles and the goal for lead is to count 100 particles. In the event that these goals are achieved in less than 8 hours, particle counting of Pb and As will be discontinued but counts of the other target metals (Cd, Zn, In, Tl, Se, Hg and Sb) will continue until the 8 hours has expired. NIST 2710 or 2711 "Montana soils" will be speciated for traceability.

Quantitative elemental analysis, primarily performed on slag or other variable, metal-bearing forms, will have precision and accuracy evaluated on counting statistics and reproducibility of NIST or other certified standards using conventional EMP methods. In general, site-specific concentrations for these variable, metal-bearing forms will be determined by performing "peak counts" on the appropriate wavelength spectrometer. Average concentrations will then be used for further calculations. Data on specific gravity will be collected from referenced databases or estimated based on similar compounds.

7.0 PERSONNEL RESPONSIBILITY

The analysts will carefully read this SOP prior to any sample examination.

It is the responsibility of the laboratory supervisor and designates to ensure that these procedures are followed, to examine quality assurance (QA) and replicate standards, and to check EDS and WDS calibrations. The laboratory supervisor will collect results, ensure they are in proper format, and deliver them to the contractor.

Monthly reports summarizing all progress, with a list of samples speciated to date with data analyses sheets (DAS), will be submitted each month.

It is also the responsibility of the laboratory supervisor to notify the contractor representative of any problems encountered in the sample analysis process.

8.0 METHODOLOGY

8.1 Sample Preparation

Grain mounts, 1.5 inches in diameter, of each sample will be prepared using air-cured epoxy. The grain mounting is performed as follows:

1) Log the samples for which polished mounts will be prepared.

2) Inspect all disposable plastic cups, making sure each is clean and dry.

3) Label each "mold" with its corresponding sample number.

4) All samples will be split to produce a homogeneous 1-4 gram sample.

   NOTE: Separate splits for perlite must be prepared.

5) Mix epoxy resin and hardener according to manufacturer's directions.

6) Pour 1 gram of sample into mold. Double check to make sure sample numbers on mold and the original sample container match. Pour epoxy into mold to just cover sample grains.

7) Use a new wood stirring stick with each sample, carefully blend epoxy and grains so as to coat all grains with epoxy.

8) Set molds to cure at ROOM TEMPERATURE in a clean restricted area. Add labels with sample numbers and cover with more epoxy resin. Leave to cure completely at room temperature.

9) One at a time remove each sample from its mold and grind flat the back side of the mount.

10) Use 600 grit wet abrasive paper stretched across a grinding wheel to remove the bottom layer and expose as many mineral grains as possible. Follow with 1000 grit paper.

NOTE: perlite samples should be mounted on glass thin sections prior to polishing. Perlite particle counts should be counted under polarized transmitted light.

11) Polish with 15 um oil-based diamond paste on a polishing paper fixed to a lap. Use of paper instead of cloth minimizes relief.

12) Next use 6um diamond polish on a similar lap.

13) Finally polish the sample with 1um oil-based diamond paste on polishing paper, followed by 0.05 um alumina in water suspension. The quality should be checked after each step. Typical polishing times are 30 minutes for 15 um, 20 minutes for 6 um, 15 minutes for 1 um, and 10 minutes for 0.05 um.

NOTE: use low speed on the polishing laps to avoid "plucking" of sample grains.

14) Samples should be completely cleaned in an ultrasonic cleaner with isopropyl alcohol or similar solvent to remove oil and fingerprints.

15) To ensure that no particles of any metal are being cross-contaminated during sample preparation procedures, a blank (epoxy only) mold will be made every 20th sample (5% of samples) following all of the above procedures. This mold will then be speciated along with the other samples.

16) Each sample must be carbon coated. Once coated, the samples should be stored in a clean, dry environment with the carbon surface protected from scratches or handling.

8.2 Point Counting

Counts are made by traversing each sample from left-to-right and top-to-bottom as illustrated in Figure 8-2. The amount of vertical movement for each traverse would depend on magnification and CRT (cathode-ray tube) size. This movement should be minimized so that NO portion of the sample is missed when the end of a traverse is reached. Two magnification settings generally are used. One ranging from 40-100X and a second from 300-600X. The last setting will allow one to find the smallest identifiable (1-2 micron) phases.

The portion of the sample examined in the second pass, under the higher magnification, will depend on the time available, the number of metal-bearing particles, and the complexity of metal mineralogy. A maximum of 8 hours will be spent per sample.

8.3 Data Presentation

Analysts will record data as they are acquired from each sample using the LEGS software, which places all data in a spreadsheet file format. Columns have been established for numbering the metal-bearing phase particles, their identity, size of longest dimension in microns, along with their association (L = liberated, C= cementing, R = rimming, I = included) (Figure 8-3). The analyst may also summarize his/her observations in the formatted data summary files.

The frequency of occurrence and relative metal mass of each metal-bearing form as it is distributed in each sample will be depicted graphically as a frequency bar-graph. The particle size distribution of metal-bearing forms will be depicted in a histogram. Size-histograms of each metal-bearing form can be constructed from data in the file.

Data from EMP will be summarized using two methods. The first method is the determination of FREQUENCY OF OCCURRENCE. This is calculated by summing the longest dimension of all the metal-bearing phases observed and then dividing each phase by the total.

Equation 2 will serve as an example of the calculation.

$$F_M \text{ in phase-1} = \frac{\Sigma\,(PLD)_{\text{phase 1}}}{\Sigma\,(PLD)_{\text{phase-1}} + \Sigma(PLD)_{\text{phase-2}} + \Sigma\,(PLD)_{\text{phase-n}}} \qquad \text{(Eq. 2)}$$

Where:
F_M = Frequency of occurrence of metal in a single phase.
PLD = An individual particle's longest dimension
$\%F_M$ in phase-1 = F_M in phase-1 * 100

These data thus illustrate which metal-bearing phase(s) are the most commonly observed in the sample or relative volume percent.

The second calculation used in this report is the determination of RELATIVE METAL MASS. These data are calculated by substituting the PLD term in the equation above with the value of M_M. This term is calculated as defined below.

$$M_M = FM * SG * ppm_M \qquad \text{(Eq. 3)}$$

Where:
M_M = Mass of metal in a phase
SG = Specific Gravity of a phase
ppm_M = Concentration in ppm of metal in a phase

The advantage in reviewing the RELATIVE METAL MASS determination is that it gives one information as to which metal-bearing phase(s) in a sample are likely to control the total bulk concentration for a metal of interest. For example, PHASE-1 may comprise 98% relative volume of the sample; however, it has a low specific gravity and contains only 1,000 parts per million (ppm) arsenic. PHASE-2 comprised 2% of the sample, has a high specific gravity, and contains 850,000 ppm of arsenic. In this example it is PHASE-2 that is the dominant source of arsenic to the sample.

Finally, a concentration for each phase is calculated. This quantifies the concentration of each metal-bearing phase: This term is calculated as defined below (Eq. 4).

$$ppm_M = M_M * \text{Bulk metal concentration in ppm} \qquad \text{(Eq. 4)}$$

8.4 Analytical Procedure

A brief visual examination of each sample will be made, prior to EMP examination. This examination may help the operator by noting the occurrence of slag and/or organic matter. Standard operating conditions for quantitative and qualitative analyses of metal-bearing forms are given in Table 8-1. Quality control will be maintained by analyzing standards and duplicates at regular intervals (Section 8.5).

The backscattered electron images will be examined using two settings: one for light-element matrices (slag or organic) and the second for heavy-element matrices (lead sulfide or lead carbonate etc.). This procedure will minimize the possibility that metal-bearing minerals may be overlooked during the scanning of the polished grain mount. The scanning will be done manually in a manner similar to that depicted in Figure 8-2. Typically, the magnification used for scanning all samples except for airborne samples will be 40-100X and 300-600X. The last setting will allow the smallest identifiable (1-2 um) phases to be found. Once a candidate particle is identified, then the backscatter image will be optimized to discriminate any different phases that may be making up the particle or defining its association. Identification of the metal-bearing phases will be done using both EDS and WDS on a EMP, with spectrometers peaked at sulfur, oxygen, carbon and the metal of concern (M). The size of each metal-bearing phase will be determined by measuring in microns the longest dimension.

As stated previously, a maximum of 8 hours will be spent in scanning and analyzing each mount. For arsenic, the goal is to count 200 particles and the goal for lead is to count 100 particles. In the event that these goals are achieved in less than 8 hours, particle counting of Pb and As will be discontinued but counts of the other target metals (Cd, Zn, In, Tl, Se, Hg and Sb) will continue until the 8 hours has expired. NIST 2710 or 2711 "Montana soils" will be speciated for traceability.

Perlite distribution will be examined under polarized transmitted light, and will be counted according to particle size and chemical constituents. Perlite particles will be sorted according to the presence of the following minerals:

Si
Si-Al
Si-Al-Fe
Si-Al-Ca-Fe

Quantitative Analyses

Quantitative analyses are required to establish the average metal content of the metal-bearing minerals, which have variable metal contents as: Iron-(M) sulfate, Iron-(M) oxide, Manganese-(M) oxide, organic, and slag. These determinations are important, especially in the case of slag, which is expected to have considerable variation in their dissolved metal content. Results will be analyzed statistically to establish mean values. They may also be depicted as histograms to show the range of metal concentrations measured as well as the presence of one or more populations in terms of metal content. In the later case, non-parametric statistics may have to be used or the median value has to be established.

Associations

The association of the metal-bearing forms will be established from the backscattered electron images. Particular attention will be paid in establishing whether the grains are totally enclosed, encapsulated or liberated. The rinds of metal-bearing grains will be identified. Representative photomicrographs of backscatter electron images establishing the association of the principal metal-bearing forms will be obtained for illustration purposes. A positive/negative, black and white film (Polaroid 55) will be used or a 128x128 (minimum) binary image in ".tif" format may be stored. Recorded on each photomicrograph and negative will be a scale bar, magnification, sample identification and phase identification. Abbreviations for the identified phases should be used. Examples are listed in Table 8-2. A final list must be submitted with the laboratory report.

8.5 Instrument Calibration and Standardization

The WDS will have spectrometers calibrated for the metal of concern, carbon, oxygen and sulfur on the appropriate crystals using mineral standards. The EDS will have multi-channel analyzer (MCA) calibrated for known peak energy centroids. Calibration will be performed so as to have both low (1.0-3.0 KeV) and high (6.0-9.0 KeV) energy peaks fall within 0.05 KeV of its known centroid.

The magnification marker on the instrument will be checked once a week. This will be performed by following manufacturer instructions or by measurement of commercially available grids or licite spheres. Size measurements must be within 4 microns of certified values.

Initial calibration verification standards (ICVs) must be analyzed at the beginning of each analytical batch or once every 24 hours, whichever is more frequent. A set of mineral or glass standards will be run quantitatively for the metal of concern, sulfur, oxygen and carbon. If elemental quantities of the ICVs do not fall within +/- 5% of certified values for each element, the instrument must be recalibrated prior to analysis of investigative samples.

The metal-bearing forms in these samples will be identified using a combination of EDS, WDS and BEI. Once a particle is isolated with the backscatter detector, a 5-second EDS spectra is collected and peaks identified. The count rates for the metal(s) of concern, sulfur, carbon and oxygen can be either visually observed on the wavelength spectrometers or K-ratios calculated.

9.0 PERSONAL HEALTH AND SAFETY

Each individual operating the KEVEX x-ray fluorescence or electron microprobe instruments will have read the "Radiation Safety Handbook" prepared by the University and follow all State guidelines for operation of X-ray equipment.

Latex gloves and particulate masks will be worn during preparation of sample cups. All material that comes in contact with the samples or used to clean work surface areas will be placed in poly-bags for disposal.

10.0 FINAL REPORT

A final laboratory report will be provided to the Contractor. The report will include all EMP data including summary tables and figures. Individual sample data will be provided on disk.

Speciation results will include: 1) a series of tables summarizing frequency of occurrence for each metal phase identified along with a confidence limit; 2) summary histograms of metal phases identified for each waste type; 3) a summary histogram of particle size distribution in each waste type; and 4) a summary of metal phase associations. Representative photomicrographs or TIFF images will also be included in the final report.

11.0 REFERENCES

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Table 2-1

Metal-Bearing Forms Found Within Western Mining and Smelting Districts

OXIDES

- Lead Oxide
- Manganese (metal) oxide
- Iron (metal) oxide
- Lead molybdenum oxide
- Arsenic Oxide
- Cadmium Oxide
- Copper Oxides
- Zinc Oxide
- Lead Arsenate
- Arsenic Trioxide
- Calcium (metal) oxide

SILICATES

- Slag
- Lead silicate
- Arsenic silicate
- Zinc silicate
- Clays

SULFATES

- Iron (metal) sulfate
- Lead sulfate
- Lead barite
- Zinc Sulfate
- Arsenic sulfate
- Copper sulfate

CARBONATES

- Lead Carbonate
- Zinc Carbonate

PHOSPHATES

- (metal) phosphates

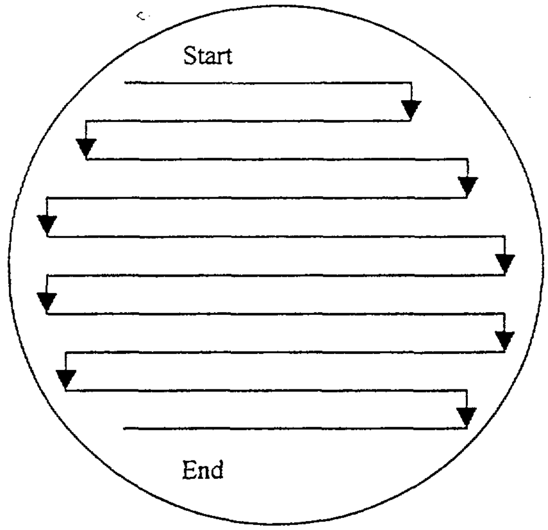
SULFIDES

- Lead sulfide
- Sulfur-containing salts
- Iron-arsenic sulfide
- Zinc sulfide
- Copper sulfides
- Copper-iron sulfide
- Cadmium Sulfide

OTHER

- Native: Lead, Copper, Cadmium, Mercury, Indium, Thallium, Selenium
- Lead/Arsenic/Cadmium/Mercury Chlorides
- Lead paint
- Solder
- Organic lead
- Lead vanadate
- Minor telluride, and bismuth-lead phases

Figure 8-2



ASSAY	______	Pb ASSAY	______
SAMPLE ID	______	LAB	______
ANALYST	______	TIME END	______
TIME START	______		

Figure 8-3

NOTES	IDENTITY	SIZE		Liberated	Inclusions	Incrusted Intergrown Cemented	Rim
		Length (um)	Area (um)				

Table 8-1

EMP Standard Operating Conditions

	WDS	EDS
Accelerating Voltage	15 KV	15-20 KV
Beam Size	1-2 microns	1-2 microns
Cup Current	10-30 NanoAmps	10-30 NanoAmps
Ev/Channel	NA	10 or 20
Stage Tilt	NA	Fixed
Working Distance	NA	Fixed
MCA time Constant	NA	7.5-12 microseconds
X-ray lines	S K-alpha PET O K-alpha LDE1 C K-alpha LDEC Zn K-alpha PET As L-alpha TAP Cu K-alpha LIF Cd L-alpha PET Pb M-alpha PET Pb L-alpha LIF In L-alpha PET Tl L-alpha LIF Hg L-alpha LIF Se L-alpha LIF Sb L-alpha PET	S K-alpha 2.31 KeV O K-alpha 0.52 KeV C K-alpha 0.28 KeV Pb M-alpha 2.34 KeV Pb L-alpha 10.5 KeV Zn K-alpha 8.63 KeV Cu K-alpha 8.04 KeV As K-alpha 10.5 KeV As L-alpha 1.28 KeV Cd L-alpha 3.13 KeV In L-alpha 3.28 KeV Tl M-alpha 2.27 KeV Tl L-alpha 10.26 KeV Hg L-alpha 9.98 KeV Hg M-alpha 2.19 KeV Se L-alpha 1.37 KeV Sb L-alpha 3.60 KeV

Table 8-2

Suggested Abbreviation for Photomicrographs

Metal-bearing Phase	Abbreviation
In	In
Tl	Tl
Hg	Hg
Se	Se
Sb	Sb
Lead Sulfide	Ga
Lead Sulfate	Ang
Lead Carbonate	Cer
Mn-(M) Oxide	Mn(M)
Fe-(M) Oxide	Fe(M)
(M)Phosphate	(M)Phos
Fe-(M) Sulfate	Fe(M)Sul
Metal Oxide	(M)O
Pb-Mo Oxide	Wulf
Slag	Slag
Metallic Phase	(M)
Metal Silicate	(M)Si
Solder	Sold
Paint	Pnt
Metal-bearing Organic	(M)(Org)
(M) barite	(M)Bar
Pb arsenate	PbAsO
Pb vanadate	PbVan
As-Sb Oxide	AsSbO
Chalcopyrite	Cp
Sphalerite	Sph
Arsenopyrite	Apy

SOP # 16 STANDARD OPERATING PROCEDURE

September 1999

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** #16

Title: In Vitro Bioaccessability (ISSI SOP No. ISSI-VBI70-10)

Total Pages 19

ISSI Consulting Group, Inc.
Author

SYNOPSIS: In vitro bioaccessability quality assurance project plan for evaluating the simplified in vitro test method.

QUALITY ASSURANCE PROJECT PLAN

FOR EVALUATING THE

SIMPLIFIED IN VITRO TEST METHOD

PREPARED FOR:

THE SOLUBILITY/BIOAVAILABILITY RESEARCH CONSORTIUM

1.0 QUALITY CONTROL REQUIREMENTS

1.1 Elements of QA/QC

The overall purpose of this project is to determine a correlation of the in vitro test method to animal study results. QA/QC requirements will be such that this correlation can be made with sufficient confidence before general use of the method can be considered acceptable.

A standard method for the in vitro extraction of soils/solid materials is specified in SOP#1, and all participating laboratories must follow the procedure set forth in the SOP to maintain consistency throughout method validation. Specific quality control procedures prior to analysis are included in SOP #ISSI-VBI70-10. These specific QC procedures involve preparation of quality control samples for analysis and are as follows (see Table 1 for summary of QC procedures, frequency, and control limits):

Reagent Blank--Extraction fluid analyzed once per batch.

Bottle Blank--Extraction fluid only run through the complete extraction procedure at a frequency of no less than 1 per 20 samples or one per extraction batch, whichever is more frequent.

Blank Spikes--Extraction fluid spiked at 10 mg/L lead and 1 mg/L arsenic and run through the extraction procedure at a frequency of no less than every 20 samples or one per extraction batch, whichever is more frequent. Blank spikes should be prepared using traceable 1,000mg/L lead and arsenic standards in 2 percent nitric acid.

Duplicate--duplicate extractions are required at a frequency of 1 for every 10 samples. At least one duplicate must be performed on each day that extractions are conducted.

Matrix Spike--The sample used for the duplicate will also be spiked prior to extraction (10 mg/L lead and 1 mg/L arsenic) to evaluate recovery of a soluble spike in the presence of test material. Matrix spikes should be prepared using traceable 1,000 mg/L lead and arsenic standards in 2 percent nitric acid. At least one matrix spike must be performed on each day that extractions are conducted.

Standard Reference Materials--National Institute of Standards and Testing (NIST) material 2711 (Montana Soil) will be used as a laboratory control sample (LCS). The LCS will be analyzed three times during the testing of solid/soil materials during method validation. These will be sent blind to each laboratory.

Control limits for these QC samples are delineated in the following discussion.

The laboratory should analyze all extracts by SW-846 method 6010B, December 1996 revision. The project-required detection limits (PRDLs) for lead and arsenic are 100 µg/L and 20 µg/L, respectively. Lead content of all soil/solid material should be sufficiently high that achieving these PRDLs by method 6010B should not be a problem.

TABLE 1. SUMMARY OF QC SAMPLES, ANALYSIS FREQUENCY, AND CONTROL LIMITS

QC Sample	Analysis Frequency	Control Limits
Reagent blank	once per batch	<25 µg/L lead <5 µg/L arsenic
Bottle blank	5%	<50 µg/L lead <10 µg/L arsenic
Blank spike	5%	85-115% recovery
Duplicate	10%	±20% RPD
Matrix spike	10%	75-125% recovery

Arsenic concentrations in samples tested for arsenic may not be high enough for method 6010B to detect above the PRDL. In those cases where arsenic is not detected in samples by method 6010B (ICP), analysis by either ICP-MS (method 6020, September 1994) or ICP-hydride (method 7061A, July 1992) will be required to reach the PRDL for arsenic.

Laboratories will follow all method requirements, and quality control samples listed in SOP #ISSI-VBI70-10 will be required.

1.2 QA/QC Procedures

Specific laboratory procedures and QC steps required include:

Calibration

Instruments will be calibrated according to method and instrument manufacturer. An acceptable calibration curve shall be one with a correlation coefficient of $\geq$0.995. At least one blank shall be analyzed for each calibration curve. The highest calibration standard shall not exceed the linear range of the instrument. At least one non-blank calibration standard shall be used for ICP (6010B) analyses, and method calibration requirements will be used for ICP-MS (6020) and ICP-hydride (7061A). All calibration standards and blanks should be matrix-matched with extracted samples.

Calibration Verification

Immediately following completion of a successful instrument calibration, an initial calibration verification standard (ICV) of known concentration and from an alternative source from the calibration standards will be analyzed. This standard should be in the mid-range of the calibration curve, and when analyzed, must be within 10% of the certified true value. If the ICV is not within 10% of the true value, the analyses will be terminated, any problems fixed, the instrument recalibrated, and the ICV rerun until a successful calibration and ICV are obtained. No samples shall be analyzed without a successful calibration and ICV. The ICV or another standard of known value at approximately mid-range shall be analyzed every ten samples (not counting QC samples) and be within 10% of its certified true value; this standard will be used as a continuing calibration verification (CCV) standard. If at any time, a CCV is not within 10% of its certified value, sample analyses will be terminated, problems fixed, the instrument recalibrated, and all samples since the last in-compliance CCV reanalyzed. The analytical run should end with a successful analysis of a CCV standard.

Calibration Blanks

Immediately following the ICV, an initial calibration blank (ICB) will be analyzed. This blank is made from contaminant-free deionized water (Type II) and should be matrix-matched with the extracted samples. No analytes of concern (lead or arsenic) should be detected in this blank. However, due to instrument and electronic noise, a positive or negative result within three times the standard deviation of the statistically derived detection limit is acceptable. If the ICB is outside this limit, the analysis shall be terminated, the problem fixed, the instrument recalibrated, an ICV analyzed with acceptable results, and an ICB reanalyzed. If problems persist, the possibility of contaminated glassware or reagents must be considered. Each ten samples and immediately after the CCVs, a continuing calibration blank (CCB) must be analyzed. The same acceptance criteria for the ICB apply to the CCB. If problems with the CCB occur, analysis must be terminated, problems fixed, the instrument recalibrated as described in the calibration section, and all samples since the last acceptable CCB or ICB reanalyzed. The analytical run should end with a successful analysis of a CCB sample.

Interference Check Samples

After the ICV and ICB standards are analyzed successfully, the laboratory shall analyze an interference check sample (ICS). The laboratory may prepare the ICS as described in the ICP (6010B) or ICP-MS (6020) methods (ICS is not required for 7061A) or purchase the ICS from commercial vendors. The ICS consists of two solutions: ICSA, which contains interferents, and ICSAB, which contains interferents and analytes. Both solutions must be analyzed as described in the method. ICSA should not contain significant amounts of analyte (arsenic or lead). If the analysis of this solution results in more than three times the standard deviation around the instrument detection limit, improper interelement or background corrections should be suspected. If this happens, the analysis should be terminated, the problem fixed, the instrument recalibrated, and ICVs and ICBs reanalyzed, followed by ICSA analysis. If the problem persists, contaminated reagents and/or glassware should also be investigated. Once the ICSA is successfully analyzed, solution ICSAB shall be analyzed. All analytes of interest in the ICSAB should be within 20% of the stated true values. If not, investigation of possible interferences should begin, and any interelement or background corrections readjusted to correct the problems. The calibration and QC standards required prior to the ICSAB must be re-analyzed (meeting all QC requirements) until a successful analysis of the ICSAB solution is obtained. Once the sequence of calibration, ICV, ICB, ICSA, and ICSAB is successfully completed, sample analysis may begin.

The ICSA/ICSAB pair must also be analyzed with acceptable results at the end of the analytical run or at the end of each eight-hour shift, whichever is more frequent.

Matrix Spikes/Duplicates

Duplicate and spike sample preparation are described earlier in this section and in SOP #ISSI-VBI70-10. Duplicate results should agree within 20% relative percent difference (RPD) as defined in method 6010B. If the RPD is greater than 25% for one duplicate set, or the average RPD for the entire study is greater than 20%, samples should be thoroughly remixed and re-extracted. Matrix spike results should be in the range 75-125%. However, because these samples have not been extensively tested by this method, the expected percent recovery is not known. The laboratory should calculate spike recovery, and if any spike results are outside the 75-125% recovery range, analyze a post-extraction spike (prepared from the previously unspiked extract). This post-extraction spike should be approximately twice the amount found in the extract.

Serial Dilution

The laboratory shall take one sample (non-spiked, non-SRM, non-QC related) and perform a 1:4 serial dilution. This dilution will then be analyzed to check for possible interference (ICP method 6010B only).

Laboratory Control Sample (LCS)

The SRM NIST 2711 will be used as a laboratory control sample for this project. Sample results for lead and arsenic should fall within acceptable control limits. These samples will be submitted blind to the laboratories, and the SBRC will evaluate results from this analysis to help determine the accuracy of test results.

Reagent Blanks/Bottle Blanks/Blank Spikes

Reagent blanks must not contain more than one-fourth of the project-required detection limits (PRDLs) for arsenic and lead (i.e., less than 5 μg/L arsenic and 25 μg/L lead). Bottle blanks must not contain arsenic or lead concentrations greater than one-half the PRDLs for arsenic and lead (i.e., less than 10 and 50 μg/L of arsenic and lead, respectively). If either the reagent blank or a bottle blank exceeds these values, contamination of reagents, water, or equipment should be suspected. In this case, the laboratory must investigate possible sources of contamination and mitigate the problem before continuing with sample analysis. Blank spikes should be within 15% of their true value. If recovery of any blank spike is outside this range, possible errors in preparation, contamination, or instrument problems should be suspected. In the case of a blank spike outside specified limits, the problems must be investigated and corrected before continuing sample analysis.

Chain of Custody/Good Laboratory Practices

All samples to be tested under this study will be shipped from Region 8 EPA under chain of custody. Each participating laboratory must sign and date the chain-of-custody form when receiving samples. The laboratory must also initial and date chain-of-custody seals, which are used to seal shipping containers and ensure that custody is not broken. Copies of the signed chain-of-custody form and chain-of-custody seals must be kept. Samples must be kept under custody while in the laboratory, and custody must be documented by each laboratory. Each laboratory must follow good laboratory practices as defined in 40 CFR Part 792 to the extent practical and possible. The goal of this project is to collect scientifically credible data to determine the usefulness and implementability of this test method, and as such, laboratory data of the highest quality must be obtained.

APPENDIX A

Extraction Test
Checklist Sheets

Extraction Test Checklist

I. Extraction Procedures

Extraction Fluid Preparation:

Date of Extraction Fluid Preparation:____________ Prepared by:_____________

Extraction Fluid Lot #:______________________

Component	Lot Number	Fluid Preparation 1L	Fluid Preparation 2L	Acceptance Range	Actual Quantity	Comments
Deionized Water		0.95 L (approx.)	1.9 L (approx.)	---		
Glycine		30.03±0.05 g	60.06±0.05g	---		
HCl [a]		60 mL (approx.)	120 mL (approx.)	---		
Final Volume	---	1 L (Class A, vol.)	2 L (Class A, vol.)	---		
Extraction Fluid pH value (@ 37°C)	---	1.50±0.05	1.50±0.05	1.45–1.55		

[a] Concentrated hydrochloric acid (12.1 N)

Extraction Test Checklist

Required Parameters:

Volume of extraction fluid (V) = 100 ±0.5 mL
Mass of test substrate (M) = 1.00 ±0.05 g
Temperature of water bath = 37 ±2 °C
Extraction time = 60 ±5 min

Extractor rotation speed = 30 ±2 rpm
Maximum elapsed time from extraction to filtration = 90 minutes
Maximum pH difference from start to finish (ΔpH)= 0.5 pH units
Spike solution concentrations: As = 1 mg/L; Pb = 10 mg/L

Date of Extraction:____________________
Extraction Fluid Lot #:____________________
Extracted by:____________________

As Spike Solution Lot #:____________________
Pb Spike Solution Lot #:____________________

Extraction Log:

Sample ID	Sample Preparation		Extraction								Filtration	
	V (mL)	M (g)	Start Time[a]	End Time[a]	Elapsed Time (min)	Start pH	End pH	ΔpH	Start Temp (°C)	End Temp (°C)	Time[a]	Time Elasped from extraction (min)
Acceptance Range	(95.5-100.5)	(0.95-1.05)	---	---	(55-65 min)	---	---	(Max = 0.5)	(35-39)	(35-39)		(Max = 90 min)
Bottle Blank												
Duplicate												
Matrix spike												

a – 24-hour timescale

II. Analytical Procedures

Analytical Batch Sequence Requirements:
The following sequence is required for analysis:
Initial Calibration
Initial Calibration Verification (ICV)
Initial Calibration Blank (ICB)
Interference Check Sample (ICSA & ICSAB) [ICP only]
10 Sample Analyses
Continuing Calibration Verification (CCV)
Continuing Calibration Blank (CCB)
10 Sample Analyses
CCV
CCB
10 Sample Analyses*
CCV*
CCB*
ICS (ICSA & ICSAB) [ICP only]

* This sequence will continue until sample analyses are complete or until one 8-hour shift is complete.

QC Requirements:

QC Sample	Analysis Frequency	Control Limits	Corrective Action[a]
Reagent blank	once per batch	< 25 µg/L Pb <5 µg/L As	Investigate possible sources of target analytes. Mitigate contamination problem before continuing of analysis.
Bottle blank	once per batch (min. 5%)	< 50 µg/L Pb <10 µg/L As	Investigate possible sources of target analytes. Mitigate contamination problem before continuing of analysis.
Blank spike	once per batch (min. 5%)	85-115%	Re-extract and reanalyze sample batch
Duplicate	10% (min. once/day)	± 20% RPD	Re-homongenize, re-extract and reanalyze
Matrix spike	10% (min. once/day)	75-125% recovery	Perform post-digestion spike
Post-digestion spike	If matrix spike is outside control limits	---	---

RPD – Relative percent difference
a – Action required if control limits are not met

Extraction Test Checklist

Calibration:

Initial calibration requirements:

- ____ Calibration standards were matrix matched.
- ____ Calibration curve correlation coefficient was ≥0.995.

Continuing calibration requirements:

- ____ All ICVs and CCVs were recovered within control limits (90–110%).
- ____ ICVs and CCVs were run in the correct sequence with the correct frequency.

Continuing calibration blank requirements:

- ____ All ICBs and CCBs did not contain Pb or As at levels outside of control limits ($\pm 3\sigma \times$ IDL).
- ____ ICBs and CCBs were run in the correct sequence with the correct frequency.

Interference check sample requirements (ICP only):

- ____ All ICSAs did not contain significant concentrations of Pb or As ($\leq 3\sigma \times$ IDL).
- ____ All ICSABs were recovered within control limits (80–120%).
- ____ ICSs were run in the correct sequence with the correct frequency.

Serial dilution requirements (ICP only):

- ____ A 1:4 dilution was performed on a non-spiked, non-SRM, non-QC sample.
- ____ The RPD was calculated to evaluate for possible interferences.

STANDARD OPERATING PROCEDURE

Date: September 1999 (Rev. # 0) **SOP No.** ISSI-VBI70-10

Title: *In Vitro* Method for Determination of Lead and Arsenic Bioaccessibility.

Total Pages 7

SYNOPSIS: This SOP describes an *in vitro* laboratory procedure to determine the solubility (bioaccessibility) of arsenic and lead in soil and other solid materials under a standardized set of test conditions. This SOP has been adapted from the method developed by the Solubility/Bioavailability Research Consortium (SBRC).

REVIEWS:

DATE	SIGNATURE/TITLE	ACTION
9/10/99	Bonita Lavelle / RPM	Approved for use at the VBI70 Site
9/13/99	WS Brattin	

IN VITRO BIOACCESSIBILITY OF LEAD AND ARSENIC IN SOIL

1.0 INTRODUCTION

When a human ingests contaminated soil, the health risk to the person depends on the fraction of the ingested chemical that is absorbed into the body. The fraction of an ingested dose of chemical that is absorbed into the body is referred to as the "bioavailability". For convenience, the bioavailability of a chemical in soil is usually described in comparison to the bioavailability of the pure chemical given in water or food. This ratio is called the "relative bioavailability" (RBA):

$$RBA = \frac{Bioavailability\ of\ chemical\ in\ test\ material}{Bioavailability\ of\ reference\ material}$$

The RBA may differ widely between chemicals and between soils, depending on a number of chemical and physical attributes of each.

The RBA of a chemical in a soil is usually estimated by studies performed using an appropriate animal model. During the period 1989–97, EPA Region VIII developed and applied a juvenile swine model to measure RBA of lead and arsenic in approximately 20 soils/solid materials (Weis and LaVelle 1991; Weis et al. 1994; Casteel et al. 1997a). However, such tests are costly and require special laboratory equipment and technical skills. For this reason, alternative methods for estimating bioavailability are of interest.

Several researchers have developed in vitro tests to measure the fraction of a chemical solubilized from a soil sample under simulated gastrointestinal conditions. This measurement is referred to as "bioaccessibility". Bioaccessibility is thought to be an important determinant of bioavailability, and several groups have sought to compare bioaccessibility determined in the laboratory to bioavailability determined in animal studies. Results obtained to date indicate that in vitro bioaccessability measurements may provide useful information on the in vivo bioavailability for lead and arsenic.

The method described in this SOP represents an in vitro method for measuring the bioaccessability of lead and arsenic in soils and other similar solid materials. The method employed was developed by the Solubility/Bioavailability Research Consortium (SBRC), based on earlier work by Imber (1993), Ruby et al. (1993, 1996), and Medlin (1997).

2.0 SAMPLE PREPARATION

All soil/material samples are prepared by drying (<40 °C) and sieving to <250 μm. The <250-μm size fraction is used because this particle size is representative of that which adheres to children's hands. Samples must be thoroughly mixed prior to use to ensure homogenization before removal of the dose material.

3.0 APPARATUS AND MATERIALS

3.1 Equipment

The main piece of equipment required for this procedure is a Toxicity Characteristic Leaching Procedure (TCLP) extractor motor that has been modified to drive a flywheel. This flywheel in turn drives a Plexiglass block situated inside a temperature-controlled water bath. The Plexiglass block contains ten 5-cm holes with stainless steel screw clamps, each of which is designed to hold a 125-mL wide-mouth high density polyethylene (HDPE) bottle. The water bath must be filled such that the extraction bottles are immersed. Temperature in the water bath is maintained at 37± 2 °C using an immersion circulator heater (for example, Fisher Scientific Model 730. The 125-mL HDPE bottles must have an air-tight screw-cap seal (for example, Fisher Scientific 125-mL wide mouth HDPE Cat. No. 02-893-5C), and care must be taken to ensure that the bottles do not leak during the extraction procedure. Additional equipment for this method includes typical laboratory supplies and reagents, as described in the following sections.

3.2 Standards and Reagents

The leaching procedure for this method uses an aqueous extraction fluid at a pH value of 1.5. The pH-1.5 fluid is prepared as follows:

Prepare 2 L of aqueous extraction fluid using ASTM Type II deionized (DI) water. The buffer is made up in the following manner. To 1.9 L of DI water, add 60.06 g glycine (free base, Sigma Ultra or equivalent). Place the mixture in a water bath at 37 °C until the extraction fluid reaches 37 °C. Standardize the pH meter using temperature compensation at 37 °C or buffers maintained at 37 °C in the water bath. Add concentrated hydrochloric acid (12.1 N, Trace Metal grade) until the solution pH reaches a value of 1.50 ±0.05 (approximately 60 mL). Bring the solution to a final volume of 2 L (0.4 M glycine).

All reagents must be free of lead and arsenic, and the final fluid must be tested to confirm that lead and arsenic concentrations are less than one-fourth the project-required detection limits (PRDLs) of 100 and 20 μg/L, respectively (e.g., less than 25 μg/L lead and 5 μg/L arsenic in the final fluid; see Table 1 in the QAPP).

Cleanliness of all materials used to prepare and/or store the extraction fluid and buffer is essential. All glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, rinsed with deionized water prior to use.

4.0 LEACHING PROCEDURE

Measure 100 ± 0.5 mL of the extraction fluid, using a graduated cylinder, and transfer to a 125-mL wide-mouth HDPE bottle. Add 1.00 ± 0.05 g of test substrate (<250 μm) to the bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity prior to adding soil. Record the volume of solution and mass of soil added to the bottle. Hand-tighten each bottle top and shake/invert to ensure that no leakage occurs, and that no soil is caked on the bottom of the bottle.

Place the bottle into the modified TCLP extractor, making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125-mL bottles containing test materials or Quality Control samples.

The temperature of the water bath must be 37± 2 °C. Record the temperature of the water bath at the beginning and end of each extraction batch.

Rotate the bottles in the extractor end over end at 30± 2 rpm for 1 hour. Record start time of rotation. When extraction (rotation) is complete, immediately remove bottles, wipe them dry and place them upright on the bench top.

Allow the bottles to stand for about 15-30 minutes to allow the soil or other test material to settle to the bottom of the bottle. Open the bottle and draw extract directly into a disposable 20-cc syringe with a Luer-Lok attachment. Attach a 0.45-μm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15-mL polypropylene centrifuge tube or other appropriate sample vial for analysis. If the total elapsed time between the end of the extraction and the time of sample filtration is greater than 90 minutes, the test must be repeated.

Measure and record the pH of fluid remaining in the extraction bottle. If the fluid pH is not within ± 0.5 pH units of the starting pH, the test must be discarded and the sample reanalyzed as follows:

If the pH has dropped by 0.5 or more pH units, the test will be re-run in an identical fashion. If the second test also results in a decrease in pH of greater than 0.5 s.u., the pH will be recorded, and the extract filtered for analysis. If the pH has increased by 0.5 or more units, the test must be repeated, but the extractor must be stopped at specific intervals and the pH manually adjusted down to pH 1.5 with dropwise addition of HCl (adjustments at 5, 10, 15, and 30 minutes into the extraction, and upon final removal from the water bath [60 min.]). Samples with rising pH values must be run in a separate extraction, and must not be combined with samples being extracted by the standard method (continuous extraction).

Store filtered sample(s) in a refrigerator at 4 °C until they are analyzed. Analysis for lead and arsenic concentrations must occur within 1 week of extraction for each sample.

5.0 QUALITY CONTROL/QUALITY ASSURANCE

Quality Assurance for the extraction procedure will consist of the following quality control samples:

Reagent Blank—extraction fluid analyzed once per batch.

Bottle Blank—extraction fluid only (no added test material) run through the complete procedure at a frequency of 1 in 20 samples.

Blank Spike—extraction fluid spiked at 10 mg/L lead and 1 mg/L arsenic (use traceable 1,000 mg/L lead and arsenic standards in 2 percent nitric acid for making spikes), and run through the complete procedure at a frequency of 1 in 20 samples.

Duplicate Sample—duplicate sample extractions to be performed on 1 in 10 samples.

Matrix Spikes--a subsample of each material used for duplicate analyses will also be used as a matrix spike. The spike will be prepared at 10 mg/L lead and 1 mg/L arsenic (spike concentrations are given for the 100-mL test fluid volume) and run through the extraction procedure (frequency of 1 in 10 samples). Use tractable 1,000 mg/L lead and arsenic standards in 2 percent nitric acid for making the matrix spikes.

6.0 CHAIN-OF-CUSTODY PROCEDURES

All test materials will be transmitted to the test laboratory under chain-of-custody seal. Once materials are received, the laboratory will maintain and record custody of samples at all times.

7.0 DATA HANDLING AND VERIFICATION

All sample and fluid preparation calculations and operations must be recorded in bound and numbered laboratory notebooks. Each page must be dated and initialed by the person performing any operations. Extraction and filtration times must be recorded, along with pH measurements, adjustments, and buffer preparation. Copies of all laboratory notebook pages must be submitted with the data package.

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US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** #17

Title: Collection and Preparation of Urine Samples for Analysis of Arsenic

Total Pages 2

APPROVALS:

ISSI Consulting Group, Inc.
Author

Stan W. Casteel 9/20/99
Study Director Date

SYNOPSIS: This SOP details the procedure for collecting and preparing urine samples for use in estimating arsenic bioavailability in test materials.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
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Study QA Officer	W. Woldade	9.16.99
ISSI Consulting Group, Inc.	W S Brattin	9/16/99

COLLECTION AND PREPARATION OF URINE SAMPLES FOR ARSENIC ANALYSIS

1.0 INTRODUCTION

Several studies suggest that arsenic as well as lead may be absorbed less extensively from soils and mine wastes than from aqueous solutions. Because absorbed arsenic is excreted mainly (about 60-80%) in urine, an excellent endpoint for monitoring arsenic exposure is the arsenic concentration in a timed urine void. The ratios of the amount of arsenic excreted in a given time period to the amounts dosed can be used to estimate the amounts absorbed over the dose range given.

2.0 URINE COLLECTION

Animals will be quartered in metabolism cages to facilitate urine collections. These cages will have a fine-mesh screen placed between the coarse-mesh bottom of the cage above and the collecting pan below to prevent fecal contamination of urine samples. The V-shaped collecting pan will be sloped so that all urine will drain to the central site where a plastic catch-container will be placed. Cage flooring will be situated to prohibit drinking-water contamination.

Urine collection will begin at either 9:00 or 10:00 AM on the days specified in the protocol, and will end 48 hours later. During the 48-hour collection period, urine will be removed from the collection pans at least twice daily and stored in a separate container for each animal. Therefore at the end of the 48-hour period, all collected urine will be located in one container and can be addressed as to the total volume of urine collected.

2.1 SAMPLE PRESERVATION

The 48-hour urine volume samples will be mixed by swirling in the collection vessels and the volume measured by transfer into a graduated cylinder. Three 60 mL aliquots of urine will be retrieved from the 48-hour urine volume samples, placed in capped plastic urine storage bottles and acidified by addition of 0.6 mL of concentrated nitric acid. One bottle will be maintained in the refrigerator as the archive sample, while the second bottle will be sent to the laboratory for arsenic analysis.

US EPA REGION 8 ARSENIC BIOAVAILABILITY STUDY

Date: September 1999 (Rev. # 0) **SOP No.** #18

Title: Preparation and Analysis of Swine Urine for Arsenic

Total Pages 7

APPROVALS:

Ed Hinderberger L.E.T., Inc.
Author

Stan W. Casteel 9/20/99
Study Director Date

SYNOPSIS: This SOP details the procedure for collecting and preparing urine samples for use in estimating arsenic bioavailability in test materials.

REVIEWS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
US EPA Region 8	CPlum	9/17/99
Study QA Officer	M Goldade	9.16.99
ISSI Consulting Group, Inc.	W J Brattin	9/16/99

1.0 PURPOSE

Bioavailability of arsenic will be evaluated for several test materials. Arsenic doses will be administered to juvenille swine on a predetermined schedule. Urine samples will be collected, digested, and analyzed for arsenic.

2.0 SCOPE

The procedures described in this SOP are for the preparation and analysis of arsenic by hydride generation. These methods were developed by the L.E.T laboratory in Columbia Missouri and are intended primarily for use in this study. They may not apply to other types of analyses.

3.0 SAMPLE PREPARATION

Transfer 25 ml. of urine to an acid cleaned 100 ml. beaker. Add 3.0 ml. of Methanol, 5 drops of anti-foam agent, 10.0 ml of 40% (W/V) Magnesium Nitrate Hexahydrate, and 10.0 ml of concentrated trace metal grade Nitric acid (HNO_3). Cover with a watch glass and place on a hot plate to reflux for 8-12 hours at 70 - 80 C. or overnight. Increase heat to 200 C and slide the watch glass back to allow faster evaporation. Heat to complete dryness (8 - 12 hours). Cover with watch glass and allow to cool. Transfer samples to cool muffle furnace and run following program. Ramp to 500 C at 1 degree/minute then hold at 500 C for 3 hours, turn off and allow to cool. Remove samples and add 5 ml. D.I. Water and 5 ml concentrated trace metal grade Hydrochloric acid (HCl). Allow to gently boil until the white residue is dissolved. After dissolving the residue cool and dilute with D.I. water to 50.0 ml. Transfer to clean labeled 2 oz. bottles.

When samples are ready for analysis they are diluted for Hydride Generation AA with a solution of 10% HCl, 10% KI, and 5% Ascorbic Acid. The samples are initially diluted l/10 or 1/5 in 1O.O ml. depending on the detection limit desired and capped. A 1/10 dilution should give a detection limit of 2 mg/L and a 1/5 should give a detection limit of 1 mg/L. Samples should set at least 30 minutes before analysis, but overnight is preferred.

4.0 APPARATUS AND MATERIALS

4.1 Equipment

The basic analysis equipment required are the Perkin-Elmer 3100 atomic absorption spectrometer (AAS), the Perkin-Elmer FIAS 200 flow injection system, the Perkin-Elmer autosampler (AS-90), computer, monitor, and printer.

4.2 Standards, Reagents, and Miscellaneous

Other materials required are acid cleaned beakers for sample digestion, hotplate, fumehoods, Class A volumetric glassware and pipets for sample dilution and standards preparation, and the following reagents:

Trace Metal Grade Hydrochloric Acid – Fisher
Trace Metal Grade Nitric Acid – Fisher
Anti-Foam Reagent
Methanol ACS grade - Fisher
Electrolytic Sodium Hydroxide - Fisher
Potassium Iodide A.C.S. grade - Fisher
1000 PPM Arsenic standard - Fisher
Sodium Tetrahyridoborate 98% - Alfa
Ascorbic Acid - Recrystalized
Arsenic Calibration Check Standard - ERA

Purity of reagents is essential in this analysis. Contamination in samples and standards is often the limiting factor in low level analysis. High purity reagents must be used to avoid introducing contaminants during sample digestion. Calibration and check standards should not be used beyond the certified expiration date. Reagents must be either analytical reagent (AR) or ultrapure grade and should be chosen based on instrument response. Ideally, peak area absorbance should be near zero or small compared to the reporting limit. Calibration standards are prepared from the 1000 ppm arsenic standard.

6.0 ANALYTICAL PROCEDURES

6.1 Instrument Setup

Turn on the Perkin-Elmer 3100, FIAS 200, AS-9O, Computer, Monitor and Printer. Make sure the quartz cell is the one used for Arsenic, if not then change to the correct cell. Use the mouse whenever possible on the computer. When Dosshell comes on the screen, double click on Perkin-Elmer 3100. On the next screen double click on the aa inst.exe icon. On the next screen, when the icons for the 3100, FIAS 2OO and AS-90 are lit, click on the MHS-FIAS box, and then double click on the AUTO icon. Click on Element file, then double click on ASTST.MEL. When the four screens are completed, click on the FIAS control box and click on the Cell On/Off box to turn on the cell. The method will probably have less drift if the cell is allowed to heat overnight at 900 deg. C; if there is a rush it can be used as soon as the temperature reaches 900.

After overnight warm up or while the cell is heating to 900 deg. C, prepare the standards in 10% HCl, 10% KI, 5% Ascorbic Acid. Prepare the following standards: 0.0, 0.2, 1.0, 5.0, 10.0, and 15.0 PPB. Prepare the ERA standard at about 5.0 mg/L for use as a calibration check standard. Turn on the EDL power supply and light the EDL Arsenic lamp and adjust the wattage to 8-10 watts. Place the Arsenic lamp in the instrument and adjust the wavelength to 193.7 with a 0.7 High Slit. After 10-15 minutes, move mouse arrow to windows and click on Align Lamps. Optimize the lamp energy using the wavelength knob and the two lamp position knobs. Click on AGE/AIC if the energy goes over 3/4 of the scale. Click on the upper left corner to close Align Lamps and move the mouse arrow to windows and click on Continuous Graphics. The absorbance reading will probably not be zero, so it will be necessary to autozero the reading. Using the three position knobs on the furnace head adjust the position to give the lowest reading. It may be necessary to autozero during the adjustments. When the best position has been found move the mouse pointer to the upper left corner and click to close the Continuous Graphics box.
Make sure that the reaction cell and reagent tubes are the correct ones for Arsenic. Change all the peristaltic tubing on the FIAS pumps. Prepare fresh 10% HC1 carrier and 0.2% NaBH4 in 0.5% NaOH. Approximately 2 liters of HCL will be needed for each liter of NaBH4.

Move arrow to Windows and click on ID/Weight Parameter. Enter all data on the samples to be run(sample number autosampler position and dilution) Up to 98 samples can be run on one autosampler load. When all the sample information has been entered and checked, move the mouse arrow to File and click on Save As.... enter the name of the file(usually the number of the first sample to be analyzed minus the 9 exp. L95080001 would be entered as L5080001) this is necessary since the file name can only have 8 characters. Click on OK to save the file with that name and then move the mouse arrow to the upper left and click to exit ID/Wt Parameter.

6.2 Calibration

Click on AS-90 Control box and enter the name and the Data File: (usually the same as the first sample to be run that day the first number). Enter ASSTD as the ID/Wt File name (this file has the assignments for the calibration, standards check, known QC check and detection limit determination). Fill all the tubes and place in the positions required by the ASSTD ID/Wt File. Place the appropriate tubes in the 10% HCl and NaBH4 solutions. Click on the FIAS Control box and start pump 2. Move the clamps into position to start pumping the reagents. Turn on the Argon, after you are sure no liquid is going to go through the tube that goes to the Hydride cell, connect it to the cell. Click on Pump 1 and position its clamp to start pumping. Make sure that the rinse(zero standard) cup has the 10% HCl, 10% KI, and 5% Ascorbic Acid zero standard. Click on the AS-90 Control box and enter 8 in the Samples to Run: line and click on the box. Click on Run Samples. The 5.00 PPB standard should be run 3 times and the absorbance values will be given in the lower left screen. If the last two values are within 5% of one another then the standardization can start. Click on Save Data On/Off and Printer On/Off(the boxes should be black if they are on) Click on Samples to Run ID/Wt box, and then on Run All. This should start the standardization. The standards run are S1 - 0.00, S2 - 5.00, and S3 - 15.00, this will calibrate from zero to 15.00 PPB. After the standardization the 5.00 standard in cup 8 will be run, if it is within 5% of 5.00 (4.75-5.25) the analysis will proceed. The next samples are a 10.00 standard and the known sample (ERA ~5.0 mg/L). If these are within the acceptable limits then the next ten samples will be the 0.2 PPB standard to determine the detection limit. Do a mean and standard deviation of the ten values (three times the standard deviation will be used as the detection limit). If the detection limit is not at least 0.1 PPB, then try to determine the problem and start the calibration over after solving the problem. The 5.00 PPb standard is run every ten samples and if it is not within the range 4.75 - 5.25 then the instrument will be restandardized and the 5.00 PPB standard rechecked, if it is still outside the range the analysis will stop.

6.2 Sample Analysis

After standardization, acceptance of the standard check samples, and detection limit, samples can then be analyzed. Move the mouse pointer to ID/Wt File: and enter the name of the file you want to run. Move the mouse arrow to reset sampler and click. Make sure that the Printer On/Off and Save Data On/Off are on. If it is necessary to turn data on again, make sure you append the data and do not write over the file. Place all the samples in the auto sampler in the positions matching the ID/Wt File:. Click on the box by ID/wt. to run the ID/wt file and then click

on Run Samples. An independent check standard (ERA ~5.0 mg/L) will be analyzed every 10 samples to verify instrument calibration (within 10% of accepted value). The instrument will check the 5.00 standard and if acceptable will then start the samples. The 5.0 standard is checked every 10 samples, and if it is not within the range 4.75 to 5.25 the instrument will be restandardized and if the 5.00 is still not in the range it will stop until the problem is resolved and restarted. Check the printer frequently to find any samples that are above 10.00 PPB, make the appropriate dilutions to be run after the other samples have been run.

7.0 DATA HANDLING

After all the samples and dilutions are run then the data can be reformatted. Move the mouse pointer to File and click on Exit to Benchtop. When in Benchtop, move the mouse pointer to Windows and click on Reformat Data, type the name of the data file and hit enter. Change the extension to AS, click on the box to it's left, then click on Header Included. To go to the next page click, on Sample, then click on Sample Position, Dilution, Mean Conc (std units) and finally on Execute Reformat. The reformatted file is now saved. Exit by moving the mouse pointer to File and click on Exit to DOS. Click on OK when told that changes will be lost to reformat and OK when asked if OK to exit software. When back in Dosshell, double click on Command Prompt. When C:\DOS> is on the screen then type cd.. and enter. Then type aa_inst\aa_files\data and enter. Type print xxxxxxxx.as(your file name) and enter. Hit enter when asked for name of list device [PRN]. This should print out your reformatted data. Next place a 3.5" disc in the computer and type xcopy xxxxxxxx.as B:. This will transfer the file to disc for use in the calculations. The data are corrected for drift using quattro, then transferred to the main quattro file for final calculation. The formula is (corrected soln. conc. X dilution X final volume)/sample volume(ml.). the units are Ng/ml. or mg/L.

8.0 HEALTH AND SAFETY

Laboratory personnel should always follow established laboratory safety procedures. All digestions using perchloric acid should be performed in the perchloric acid fumehood.

9.0 QUALITY CONTROL

Quality control samples will be prepared at the following frequency:

Preparation Blank - 1/20 samples
Duplicate - 1/10 samples
Matrix Spike - 1/10 samples
NIST (#2670) Toxic Metals Freeze Dried Urine (Laboratory Control Standard) - 1/20 samples.

Contamination should be at a minimum in the blank samples and in no instance should it exceed the instrument detection limit. If contamination exceeds the instrument detection limit, the source of the contamination should be eliminated, the affected samples redigested and reanalyzed.

The difference between duplicate samples should not be greater than 1 mg/L if the values are <10 mg/L during the analysis run. At values ≥ 10 mg/L, the duplicate values should have a relative percent difference (RPD) of 20% or less. The RPD is calculated using the following formula:

$$RPD = \frac{S - D}{\frac{S+D}{2}} \times 100$$

Where:

S = Original sample value.
D = Duplicate sample value.

Matrix spike recovery should be in the range 85-115% and the LCS recovery should be within the established control windows.

If duplicate precision, matrix spike, or LCS control limits are not met for a sample batch, potential problems should be investigated and solved. If sample preparation is found to be the problem, the affected samples should be redigested and reanalyzed. If the analytical instrumentation is the problem, the affected samples and QC should be reanalyzed once the problem is solved.

The calibration check standard (ERA ~ 5.0 mg/L) will be analyzed every ten samples. If this standard is not within $\pm$ 10% of the true value, the analysis will be stopped, any problems solved. The instrument will be recalibrated and all samples since the last good calibration check reanalyzed.

The data, calculations, quality control and reports are checked by the Quality Assurance Officer before a final report is released.